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GENES AS PHYSIOLOGICAL AGENTS[1]

GENERAL CONSIDERATIONS

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MY function in this symposium is to sketch in a background for the concrete topics to be presented by the others.

It is appropriate to consider first what a gene is, especially as it has been questioned recently whether the concept has not outlived its usefulness (Goldschmidt, 1940). A gene is primarily, of course, merely a hypothetical entity postulated to make sense out of the results of breeding experiments. The time has long passed, however, since there has been need for any hesitation in identifying the mendelian units with regions of concrete chromosomes. A mendelian gene is a block of self-duplicating chromosomal material that is not divided as far as observed by the process of crossing over or by the chromosome breakage, associated with rearrangement.

There is no necessary implication that the gene is an absolute entity or that it is the smallest unit capable of self-duplication. A study of the effects of multiple alleles suggests some degree of physiological unity as the rule, but there are interesting exceptions which suggest that the essential pattern may often be replicated along the chromosome and that such "repeats" within the gene tend to drift apart in complementary fashion, by a well-

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known evolutionary mechanism, to become at length distinct genes, separable by crossing over and chromosome breakage (*cf.* Lewis, 1941; Oliver and Green, 1944). Estimates of average gene size, on the hypotheses that all the material in a Drosophila sperm head is genic, give figures of the order of 10^{-16} grams (Morgan, 1922; Muller, 1929) corresponding to a molecular weight of 6×10^7 and thus some thousand fold that of a typical small protein, though comparable to the so-called giant molecules of certain proteins (hemocyanin) and the crystallizable nucleo-proteins of viruses (Stanley, 1943). Most investigators find the condensed chromosomes of sperm cells to consist almost wholly of basic proteins (protamine or histone) combined with highly polymerized desoxyribose nucleic acid (*cf.* Mirsky and Pollister, 1943).

It has been maintained that both of these are too simple in chemical composition to provide an adequate basis for the practically infinite diversity of genes. The possibilities of stereoisomerism are, however, almost unlimited. Moreover, there is now evidence of a direct sort that desoxyribose nucleic acid actually can transmit from one organism to another the potentiality for indefinitely continued synthesis of itself and also of a chemically unrelated specific substance. Avery, McLeod and McCarty have recently obtained an extract from one specific type of pneumococcus, apparently consisting of pure polymerized desoxyribose nucleic acid (molecular weight about 500,000), which can transform unencapsulated cells from another type into the type from which the extract was obtained. The desoxyribose nucleic acid not only conveys the capacity to produce the specific polysaccharide on which type specificity depends but also the capacity to produce more of itself. As to the protein constituent, the spacing of amino acids in a polypeptide chain is practically the same as that of nucleotides in nucleic acid (3.34Å, Astbury, 1941) and there is the possibility of corresponding specificities based on stereoisomerism. It should be added that Stedman and Stedman (1943) have

recently reported the presence of large amounts of a more complex protein of the globulin type in fish sperm.

The essential pattern of the gene can hardly be more than 2 dimensional to permit synthesis of a duplicate, except that, as noted, patterns that are in some sense complementary may be represented in a nucleic acid and a protein layer. It has been maintained that the essential pattern of the gene can be represented only once or at most twice in the direction of cleavage of the chromosome to account for the usual lack of mosaicism of mutant individuals, derived directly from x-rayed sperm in Drosophila (Muller, 1928, 1929; Timofeeff-Ressovsky, Zimmer and Delbrück, 1935). But there may be any number of parallel lamellae provided that these interact in such a way that a mutational change in one is either restored rapidly to the type of the others, or spreads through them all, a process which might conceivably occur from the same mechanism as involved in the duplication process. Demerec (1928) has described an exceptional case in *Drosophila virilis* in which an influence of this sort seems to extend to a particular mutable gene (reddish-a) from any stable allele (type, yellow or stable reddish) in the homologous chromosome. Reddish-a is stable except in such heterozygotes. (It may be noted that stable reddish and yellow may both be like type in the part of the pattern affected in reddish-a.) A gene may perhaps be comparable to a little crystal with many repetitions of molecular pattern which, however, tend to become differentiated except in the direction of cleavage.

In this diagram (Fig. 1), I have attempted to indicate the relation of genes to characteristics at successive levels of organization. Most people seem to be born preformationists. They tend to take for granted a separate heredity for each part of the adult organism. The treatment of homology in comparative anatomy often seems to have this implication. Even physiologists sometimes attribute a character partly to physiological factors, partly to heredity, as if heredity could operate by some sort of

sympathetic magic, independently of physiological channels. The attitude of physiological genetics is that characters are determined 100 per cent. by physiological processes, but that genes are the ultimate internal physiological agents.

Genes can be thought to act directly only on the metabolic processes in the protoplasm immediately surrounding their positions in the cells which contain them. They

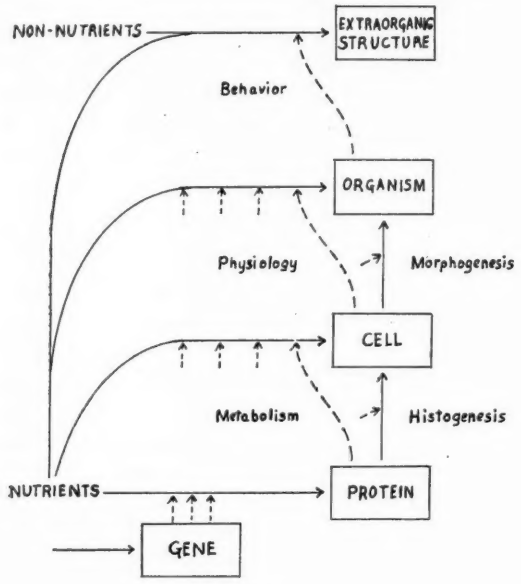

FIG. 1.

can affect morphological characters only by determining one or another reaction of cells to local conditions. The relation of genes to organic structure does not differ essentially from that to hereditary extraorganic structure as of webs of spiders, nests of termites, wasps, birds, etc. As indicated in the diagram, a gene is related to a character through a chain of processes in which behavior at each level determines structure at the next, while this in turn gives a secure basis for behavior at the higher level (Wright, 1934a).

The most characteristic physiological action of a gene is the synthesis of another gene like itself. Let us consider what this involves under the prevailing conceptions of biochemistry. The reversible transformation of glucose into lactic acid ($C_6H_{12}O_6 \rightleftharpoons 2C_3H_6O_3$) has been shown to require about a dozen steps, each of which involves a separate enzyme system. To suppose that a highly specific giant nucleo-protein molecule is formed from nutrients by such a step-by-step process seems intolerably complex, even making due allowance for repetitions and for the possibility of catalytic combinations of successively larger blocks. I think that most geneticists have long agreed that there must be a short cut. The gene must somehow act as a model on which a daughter gene is formed as a whole. (Alexander and Bridges, 1928; Koltzoff, 1928; Haldane, 1935, etc.). This is not a mere desire for simplicity. The high degree of autonomy of genes and their capacity to duplicate as of the new sort after mutation are hardly compatible with any step-by-step process. Autonomy, is, to be sure, a relative matter. The enzyme trypsin acts as an autocatalyst in transforming the substance trypsinogen into more trypsin. But trypsinogen is probably as complex a molecule as trypsin. At the other extreme is the autonomy of a sulfur bacterium or a green plant which produces its own kind of material almost from the elements. We ourselves at a higher level of organization are intermediate in this scale and so apparently at a much lower level are genes and viruses. If the synthesis of a given gene depended on the prior synthesis of specific substances of increasing complexity, catalyzed by products of a succession of other genes, to which the parent gene contributes merely a final touch, we would expect to find that mutations at any given locus would bring about mutational changes at many other loci. A few cases are indeed known in which replacement of one gene by an allele tends to bring about mutation of another gene (Demerec, 1941; Rhoades, 1941), or of many others (Demerec, 1937; Mampell, 1943).

These cases show that genes are not always completely autonomous. Genes and viruses are indeed all probably dependent with respect to energy requirements. The rule, however, is a very high degree of autonomy with respect to constitution. It is difficult to avoid the conclusion that the pattern of forces emanating from a gene somehow tends in the living cell to arrange simple molecules on its surface in such a way as to duplicate its molecular pattern. The possibility of a reciprocal rela-

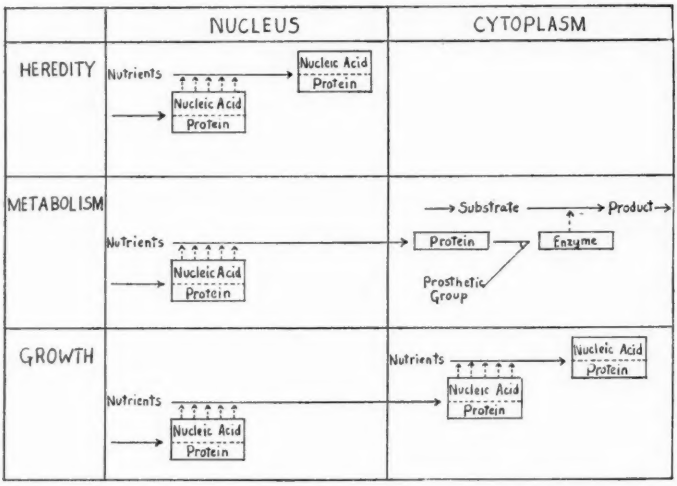

FIG. 2.

tion between steric patterns of polynucleotide and polypeptide chains has been noted. This process is represented diagrammatically in the upper portion of Fig. 2 for comparison with similar models for other aspects of immediate gene physiology.

There is, of course, no doubt that numerous chemical transformations of small molecules occur in cells in the conventional step-by-step fashion. It is quite possible that the genes themselves may function as catalysts for intranuclear reactions. But in general such processes are carried out by enzymes which are certainly not the genes themselves. This is obviously true of the digestive enzymes which act outside cells and also of those which

have been located in mitochondria and microsomes in the cytoplasm (Lazarow, 1943).

The enzymes as far as known are either pure proteins or proteins combined with a prosthetic group (which the organism in some cases is unable to synthesize and must obtain from its food as a vitamin). Assuming that the enzymes are produced by gene action, we are again confronted by the question whether this is a process of step-by-step synthesis dependent in each case on many genes or is one of production as a whole by a single gene presumably as at least a partial duplication of the gene pattern (Wright, 1927, 1941). The latter concept is represented diagrammatically in the middle of Fig. 2.

Sporadic examples of apparent one-to-one relation between gene and enzyme or elementary metabolic process have been known almost since the rediscovery of mendelian heredity. It has remained for the Stanford group, led by the geneticist Beadle and the biochemist Tatum, to develop a method of attacking the genetics of processes at this level in a systematic fashion in one convenient organism, the mold Neurospora. A member of the Stanford group, Dr. Horowitz, will present in this symposium some of the extraordinarily interesting results which have been obtained (*cf.* Tatum and Beadle, 1942; Beadle and Coonradt, 1944).

Another fundamental physiological process, the relations of which to genetics remain to be clarified, is growth. We must distinguish two very different possibilities for gene action. Most of the many genes, known to differentiate large and small varieties, doubtless act mainly by modifying conditions which affect the rate of growth. But growth, as the multiplication of proteins, specific to the species and individual, is a process of the same essential nature as heredity itself. Again, we have the question whether the protein molecules are produced by step-by-step synthesis by specific polyptidases (*cf.* Fruton, 1941) or are produced as wholes on a model already present. If we assume the latter and postulate that par-

ticular genes are the models for each kind of protein, we encounter the difficulty that while each gene produces only one daughter gene between cell divisions, it would probably have to produce millions of protein molecules to account for doubling of the cell. This difficulty is partially circumvented by postulating that the chromosomes contain many representatives of any gene that produces an abundant kind of protein molecule. It has, indeed, been suggested that the two kinds of chromatin that are recognized, euchromatin and heterochromatin, differ in function in that the former produces physiological agents, the enzymes, which are effective in minute amounts, while the latter produces substances needed in bulk, such as nucleic acid and tissue proteins (Caspersson, 1941a; Schultz, 1941; Darlington, 1942).

The difficulty would be further obviated, if we suppose that there are proteins in the cytoplasm that can function as models on which more proteins of the same sort can be formed. It is known that large amounts of ribonucleic acid, closely related to the desoxyribose nucleic acid found only in chromosomes, are present in rapidly dividing cells (Caspersson, 1941b). This nucleic acid is located in the microsomes and mitochondria (Lazerow, 1943; Claude, 1943). Claude has suggested that these are self-duplicating bodies.

It may be urged against the idea of such plasmagenes that there is no growth whatever in enucleate cells, but this may be due to other conditions. A more serious difficulty is the sparsity of evidence for cytoplasmic heredity (East, 1934; Correns, 1937). There is some evidence in plants but largely in relation to particular cytoplasmic constituents, the plastids. There is virtually no unambiguous evidence from higher animals. The complete transformation of species hybrids to the type of the paternal species by a few generations of backcrossing to the latter (always as male parent) in cases in both plants (Gärtner quoted by Mendel, 1866; Goodspeed and Clausen, 1917) and in animals (Stern, White and Spencer,

1944) tends to indicate that transmission outside of the nucleus is at best of casual rather than of fundamental importance.

A possible indicator of the site in the germ cells of the postulated models for protein multiplication may be obtained from the mode of inheritance of immunological specificity. The evidence from the genetics of transplantation reactions (Little and Strong, 1924) and of blood cell and serum antigens (Wiener, 1943; Irwin and Cole, 1943; Cumley, Irwin and Cole, 1943) indicates determination of each kind of specificity by a single mendelian gene. The only exceptions are the hybrid substances found by Irwin and associates in certain species crosses, and these are exceptions only in that more than one gene is involved.

One possibility of reconciling these results is the hypothesis that the cytoplasm of the egg transmits self-duplicating nucleo-proteins that lack antigenic specificity, that specificity is supplied in somatic cells by a combination with haptens produced by genes, and that this combination then behaves as a compound plasmagene (*cf.* Wright, 1941). Another (bottom of Fig. 2) is that the model nucleo-proteins are produced as wholes by duplication of special genes, and that they retain the genic property in the cytoplasm, but subject to decay, at least along the germ line. Small non-duplicating protein molecules, including enzymes, may be products of such decay. There is some evidence, not only for a process of self-duplication in the cytoplasm, but also for its decay, in the cytoplasmic lag of ciliate protozoa described by Jennings (1940) and his associates. They find gradually decreasing cytoplasmic control after a cross involving characters, that in the long run are completely determined by mendelian genes. There is more persistence (up to 36 generations) than can be accounted for by passive transmission without multiplication. The entire category of Dauermodifikationen of Jollos involving persistence for much greater periods, may come here, but the mode of inheritance is not clear.

The common denominator of these interpretations of heredity, control of metabolism and growth is dependence on specific nucleo-proteins, the genes, characterized by the property of synthesizing substances like themselves. It is possible that all gene action traces to this one process and that proteins are formed in no other way.

As in the case of growth, the problem of the nature of tissue differentiation can easily be dismissed by the geneticist as outside his conventional field. If, however, we consider the cell as an organism, a genetic problem is clearly presented in the origin of differences that persist more or less indefinitely under neutral conditions, as for example in repeated transplants of a particular kind of tumor cell.

A geneticist, on recognizing that there is a genetic problem here, naturally thinks first of the possibilities of nuclear change. There are two alternatives under this head. Nuclear differences may arise from the sorting action of non-equational mitosis, as postulated by Weismann, but now generally rejected for good reasons, or they may arise from mutations which are induced in orderly fashion under special local conditions and which thereafter stabilize an otherwise labile developmental pattern. There is evidence for a class of unstable genes in which mutation is under some degree of local control (*e.g.*, miniature-a of *Drosophila virilis* as described by Demerec, 1941) but it is a rather long step from these to orderly differentiation. Moreover, irreversible differentiation within uninucleate cells as in eggs of ctenophores and tunicates can hardly have its origin elsewhere than in the cytoplasm. The usual and more probable view is that the differences in cell heredity that arise within a multicellular organism are cytoplasmic.

Again, we may distinguish two alternatives. Persistence may be based on interactions among constituents which make the cell in each of its states of differentiation a self-regulatory system as a whole, in a sense, a single gene, at a higher level of integration than the chromo-

somal genes. On this view the origin of a given differentiated state of the cell is to be sought in special local conditions that favor certain chains of gene-controlled reactions which cause the array of cytoplasmic constituents to pass the threshold from the previous stable state to the given one.

There is no doubt considerable truth in this idea, but the stability of the changed state certainly becomes easier to understand if it be postulated that there are self-duplicating materials within the cell which can become modified chemically and multiply as of the new sort. The second

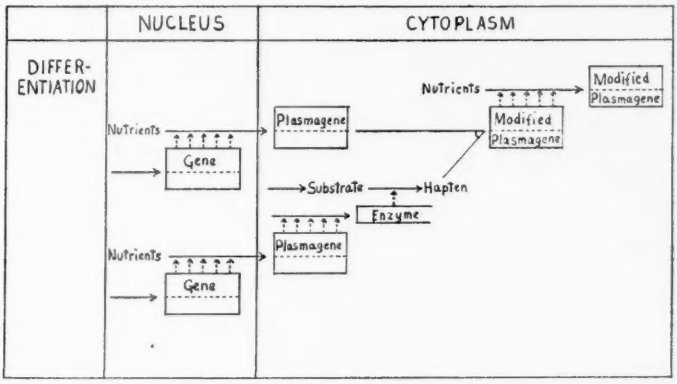

FIG. 3.

alternative is thus controlled mutation of plasmagenes. Fig. 3 illustrates this idea in relation to a plasmagene derived from a nuclear gene and subsequently elaborated by the addition of chemical groups which become available under special local conditions. The hapten may come from outside the cell, an inductor or a hormone, for example, or as indicated in the diagram, it may be a product of a chain of gene-controlled reactions initiated under the local conditions. There could be similar gene-controlled elaboration of plasmagenes which, in their main pattern exhibit cytoplasmic heredity even along the germ line (chloroplasts, for example, in which, in maize, Rhoades (1943) has found evidence of gene-controlled mutations). Dr. Sonneborn (1943) has made some re-

markable experiments on the relations between well-established mendelian genes of Paramecium and cytoplasmic components that are partially autonomous in the sense that, while dependent on specific genes for persistence and multiplication, they can not be produced *de novo* by these or any other genes. These results, wherever they may lead, are obviously of the greatest significance for these fundamental questions of physiological genetics. Dr. Sonneborn will discuss developments of this subject in the third paper.

The idea of differentiation of the cell as a self-regulatory system as a whole and that of stabilization of a particular mode of differentiation by means of the elaboration of self-duplicating entities within the cell are not incompatible. Something of the same sort appears at a still higher level in the multicellular organisms. We have noted already the impossibility of any one-to-one relation between gene and character at this level. In fact, each character is always affected by many genes as well as by environmental factors, and each gene in general has multiple effects. No qualitative distinction can be made between abnormalities due to gene replacement and ones due to special environmental treatment (the phenocopies of Goldschmidt, 1938). Organs and, after a mosaic phase of development, the organism as a whole, have very considerable self-regulatory power. The kinds of deviations from normal that are possible at any stage are rather limited. Any disturbance, whether genic or environmental, that surpasses the threshold of adaptability of the organism, or some part of it, calls forth one or another of these patterns of deviation. The apparent extreme localization of the effects of particular genes in certain cases turns out to be a matter of thresholds in the organism not of preformed rudiments in the germ plasm. Gene and environment are alike modifiers of a pattern residing in the whole. But the genes, while individually mere modifiers, constitute an array of physiological agents, selected through a long evolutionary his-

tory that somehow determines the self-regulatory pattern of the whole (*cf.* Wright, 1934b).

It was often asserted a few years ago that the mendelian genes determine only superficial individual differences, while the fundamental plan of development is cytoplasmic in its heredity. The demonstration that the commonest class of determinable mutations is that of lethals tends to refute this view. This was not entirely satisfactory, however, without knowledge of how these lethals act. Dr. Poulson (1940) has made very illuminating studies of such development as occurs in the eggs of Drosophila in which one or another portion of a chromosome is visibly deficient. Our last paper will deal with this important line of evidence on the genes as physiological agents.

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GENIC CONTROL OF BIOCHEMICAL REACTIONS IN NEUROSPORA

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THE task of physiological genetics is that of describing gene action in chemical terms. Any such description implies, in the first place, knowledge of the chemical structure of the gene, and, in the second place, an understanding of the relationship between this chemical structure and its cellular environment. Already the first rough outlines of gene structure have been drawn. Terms such as nucleic acid, polypeptide chain, prosthetic group and crystalline virus have by now found comfortable places in the genetical vocabulary. It is the second aspect of the general problem that is the subject of the discussion this afternoon, however, and more particularly, that phase of it dealing with the role of the gene in the physiology of the organism.

There is recorded in the literature a sufficient number of instances of mutations affecting the normal biochemistry of the organism to indicate that the gene exercises an important function in metabolism. The loss, through gene mutations, of the ability to form coat color pigments in mammals (Wright, 1941) and eye color pigments in Drosophila (Ephrussi, 1942) has, in particular, been investigated. Genic control of various processes involved in the production of flower pigments has also been extensively studied (Lawrence and Price, 1940). One of the clearest cases of genic control of a more or less definable chemical reaction, and also the first one to be recorded, is in the disease of man known as alcaptonuria (Garrod, 1923). Here the ability to oxidize homogentisic acid is lost, and large quantities of it are excreted in the urine. The genealogies of alcaptonurics indicate that they differ from the normal by a single recessive gene.

Studies such as these have been of the greatest value in furthering our knowledge of the gene as a physiological

agent. It is evident, however, that the relatively few investigations which have so far been made into the biochemical aspects of gene action barely touch the surface of a subject which is a very large one indeed. In particular, two questions have remained for the most part unanswered: first, do genes control any of the *essential* biochemical activities of the cell? And second, if such control exists, at precisely what points in the complex system of reactions do genes exert their influence? Answers to these questions should prove useful not only in evaluating the importance of genes as biochemical agents, but in furthering the attack on the fundamental nature of gene action.

Since the mutation of a gene controlling a vital metabolic function would be expected to be lethal, it is essential, in confronting this problem, to devise a method for analyzing lethal mutations, while at the same time selecting only those mutations for analysis which readily lend themselves to biochemical investigations. These rather severe restrictions were met by Beadle and Tatum (1941) in a procedure for the systematic detection of biochemical mutants in the ascomycetous mold, Neurospora. In the course of this work, several hundred mutations affecting the ability of the mold to carry out vital biochemical reactions have been obtained.

Neurospora is in several ways particularly favorable material for biochemical-genetical investigations of the type to be described. Because of its simple nutritional requirements it is easily kept in pure culture on synthetic media. As a result of the recent elucidation of the structure of the biotin molecule by du Vigneaud and co-workers (1942), shortly followed by its synthesis at the Merck laboratories (Harris, Wolf, Mozingo and Folkers, 1943), it is now possible to grow Neurospora on a completely defined medium. Also advantageous is the high growth rate of the mold, which makes possible enzyme studies and chemical isolations where large amounts of starting material are often required. On the genetical side, the organism—which is heterothallic—presents several

notable features. In the first place, the vegetative cells are haploid, so that complications from dominance do not ordinarily arise. In the second place, all the products of meiosis are preserved and can give rise to viable cultures. What is more, they occur in the ascus in a linear order which permits the identification of sister and cousin nuclei. Finally, the life cycle of Neurospora is short—about 12 days. We are indebted to Dodge (Shear and Dodge, 1927) and to Lindegren (1942) for much of our knowledge of the life cycle and genetics of this organism.

The method used in our laboratory for the production of mutant strains is briefly as follows: asexual spores are irradiated with high dosages of either ultraviolet light or x-rays—up to 50,000 r units in the case of x-rays. The irradiated spores are applied to wild-type protoperithecia of the opposite mating type. Sexual fusion takes place, followed by the production of ascospores. The ascospores, which are genetically homogeneous, are isolated at random to a medium made as complete as possible in vitamins, amino acids and other growth substances. After the culture has become established on this medium a transfer is made to a medium containing the minimum essentials for growth of wild type Neurospora—sugar, salts, inorganic nitrogen and biotin. If normal growth occurs on the minimal medium, the culture is considered to be wild type and is discarded. If growth fails to take place, a mutation is assumed to have been induced blocking the synthesis of an essential substance which was present in the complete medium. By a series of routine tests the identity of the missing substance can usually be determined. The strain can then be outcrossed to determine whether the lost synthesis is associated with a single gene.

To date approximately 80,000 single spore isolations have been made in our laboratory. Of these about 500 have given rise to mutant strains which are unable to carry out an essential synthesis. An unknown percentage of these are recurrent mutations of the same locus.

Probably something of the order of 100 mutant genes controlling vital syntheses have been detected.

The majority of the mutants which have so far been obtained are characterized by loss of the ability to synthesize either a vitamin or an amino acid or a nucleic acid component. Mutants for seven B-complex vitamins and possibly twelve amino acids are now known (Table 1). In the latter group are nine of the amino acids recognized as essential for the rat, dog, and human being. Aside from their theoretical interest, a number of these mutants have found practical application in bioassay work. The growth rate of each mutant is a function of the concentration of the substance in which it is deficient. By measuring the dry weight of mycelium produced during a specified growth period, or the rate of progression of the mycelial frontier over an agar surface, it is possible to obtain an estimate of the concentration of the specific substance in the medium. Bioassays using Neurospora mutants have been worked out for choline (Horowitz and Beadle, 1943), p-aminobenzoic acid (Thompson, Isbell and Mitchell, 1943), inositol (Beadle, in press), pyridoxin (Stokes, Larsen, Woodward and Foster, 1943), and leucine (Ryan and Brand, 1944). One of the advantages of Neurospora bioassays, as compared with other methods, is in the specificity of the response. This results from the fact that each strain differs in only one respect from the wild type. In this way, genetics has made an unexpected contribution to the science of nutrition.

It should be noted that the list of biochemical mutations represents only a fraction of those which must occur, but which are not picked up in the screening tests. The number of mutations found depends, of course, on the criteria of selection. In our procedure only lethals are selected; mutations affecting non-vital processes are not detected unless they produce morphological effects. In addition, much depends on the completeness of the "complete" medium. Even the best artificial medium can not contain all the substances of biological significance, and in any case mutations affecting the synthesis of unstable mole-

cules or of molecules which are unable to penetrate the cell wall would go undetected. Thus, the kinds of biochemical mutations so far found in Neurospora reflect in very large measure the limitations of the selection process.

On several occasions the mode of selection has been changed from that outlined above with results that indicate that the field of interesting mutations is far from exhausted. Thus, for a time, a medium was used in which fats formed the sole carbon source. Wild type Neurospora can use fats and certain fatty acids as its source of carbon and energy, in place of the usual carbohydrates. Mutants which have lost the ability to utilize fats will not grow on the fat medium. Two such mutants were found. Preliminary growth experiments in which fatty acids were used have indicated that one of these strains is unable to metabolize any of the fatty acids tested, while the other can utilize saturated, but not unsaturated fatty acids (Horowitz, unpublished). Another special medium we have tried is one in which nitrate forms the sole source of nitrogen, as distinguished from the ordinary ammonium-containing medium. The medium was designed to detect mutants unable to reduce nitrate to ammonia. Several such mutants have been picked up. Beadle (unpublished) has investigated one of these and has found that it grows normally on nitrite or on ammonia, but not on nitrate.

Still other kinds of selection are based on the inability of some mutants to grow throughout the entire range of temperature or pH in which the wild type is viable. Mitchell and Houlahan (unpublished) have recently been investigating a strain which grows normally at 25° C., but which entirely fails to grow at 32°, although the wild type range extends to well over 35°. Mitchell has found that the mutant will grow normally at high temperatures if the medium is supplemented with a cell-free extract of yeast or of Neurospora mycelium. The active substance has been isolated from a mycelial extract and identified as adenine. Here, then, is a mutant in which the syn-

thesis of an essential cell constituent takes place only in part of the normal temperature range of the species.

The question of the inheritance of these biochemical characters must now be considered. Because of the convenient nuclear arrangements which have already been mentioned—the haploid mycelium and the preservation in the ascus of all the products of meiosis—the mode of inheritance is very easily studied in Neurospora. A good eye and a steady hand for microdissection are the chief qualifications. Statistical analysis of large numbers of progeny is not necessary, as in higher organisms. The mechanism of meiosis is such that in a cross between two strains differing by a single gene, four spores in each ascus will carry one allele, while the remaining four spores will carry the other allele. The arrangement of the four pairs within the ascus depends on whether segregation of the gene has occurred at the first or the second meiotic division, as shown by Lindegren (1942). First division segregation results in two sets of four identical spores, while second division segregation results in alternating pairs. Second division segregation occurs when a crossover has taken place between the gene and the centromere, so that the proportion of asci showing second division segregation is a measure of the distance of the gene from the centromere.

A genetic analysis of all the strains has not yet been made. But in the cases which have been so analyzed it has been found with few exceptions that only single genes are involved. In other words, the mutation of a single gene can block the synthesis of an essential cell constituent or prevent the utilization of a normal metabolite. The possibility that in some cases we are dealing with closely linked doubles cannot, of course, be entirely eliminated.

In line with current concepts, the biochemical effects of mutations are most easily interpreted as resulting from inactivation of the gene. The wild type allele performs some essential function which is lost when the structure of the gene is changed. In diploid organisms mutations are therefore usually recessive to wild type, since a gene

which does nothing is less apparent than a gene which does something. In Neurospora, the test for dominance can not be made in the usual way, because of the ephemeral character of the diploid generation. The test can be made in another way, however. When vegetative fusion of hyphae from two different strains is allowed to take place, a situation resembling diploidy results, in that the cells will now contain haploid nuclei from two different sources. The phenotype of the heterocaryon will be determined by the two haploid sets of chromosomes it contains, and dominance can thus be studied. In all the cases we have examined, wild type is dominant, in agreement with expectation. Thus, if a heterocaryon is made from two mutant strains, each differing from wild type by a different gene, the composite organism will be phenotypically indistinguishable from wild type, since wild type alleles are present for all genes. A study of a number of such heterocaryons involving both biochemical and morphological characters of Neurospora has been made by Beadle and Coonradt (1944). They have proved that two kinds of nuclei actually occur together in the same mycelium. They also showed that the method of heterocaryons can be used as a test for allelism, since an increased growth rate in the heterocaryon, as compared with the individual components, is to be expected only if a full complement of wild type alleles is present. This can not occur if the component strains contain mutations of the same gene.

Perhaps the most interesting aspect of the Neurospora work from the point of view of biochemical genetics and of general biochemistry concerns the relation of individual genes to individual reactions. Most of the types of mutations listed in Table 1 have occurred repeatedly. For example, single gene mutations blocking the synthesis of arginine have occurred over 30 times, lysine 40 times, choline 4 times, methionine well over 50 times. Tests for allelism either by the method of heterocaryons or by crossing have shown that a fair percentage of the mutations within any one group are mutations of different single genes, the

remainder being recurrences. Each synthesis is therefore under the control of a number of different genes of which the mutation of any one is sufficient to block the synthesis. The possibility immediately suggests itself that each gene involved in a particular synthesis controls one step in the chain of chemical reactions leading to a final product. The problem thus becomes the essentially biochemical one of determining the exact point of interference of a particular mutation in the normal metabolism of the organism. The problem would be an easy one

TABLE I

VITAMINS AND AMINO ACIDS FOR WHICH MUTANTS OF NEUROSPORA ARE KNOWN

Thiamin	*Arginine
Pyridoxin	*Lysine
p-Aminobenzoic acid	*Leucine
Pantothenic acid	*Isoleucine-valine
Inositol	*Methionine
Nicotinic acid	Cystine
Choline	*Tryptophane
	Proline
	*Threonine (?)
	Serine (?)
	*Phenylalanine (?)

* Indispensable for the young rat. (?) Tentative classification.

if the biochemistry of the mold were completely known. But of course it is not completely known, and especially obscure are the reactions by which vitamins and amino acids are synthesized in living systems. Nevertheless, we have been able in several cases to unravel enough of the biochemistry of the organism to show that, in fact, a one-to-one relation exists between gene and chemical reaction. Indeed, this relationship is so specific that it has been possible to reverse the problem and to use mutants in the study of the biochemistry of the organism.

Two general methods have been found to be especially useful in approaching this problem. The first method can be illustrated by a study carried out by Srb and Horowitz (1944) on the genic control of arginine synthesis in Neurospora. At the time the investigation was started,

15 mutant strains requiring arginine were known. These strains were normal in every respect except that little or no growth occurred in the absence of external supplies of arginine. Tests for allelism showed that of the 15 strains, 7 were genetically different and 8 were recurrences at one or another locus. If the original hypothesis were correct—namely that each gene controls a different chemical reaction—it should be possible to replace arginine in the medium by suitable precursors of arginine, and it should be possible to show that a different set of precursors is available to each mutant. Information already existed in the biochemical literature indicating that two of the precursors of arginine in the animal body were the amino acids ornithine and citrulline. These were therefore tested for their ability to replace arginine in the growth of the seven mutants. It was found that four of the mutants are able to utilize ornithine and citrulline, as well as arginine; two of them can utilize citrulline and arginine, but not ornithine; while one specifically requires arginine for growth.

These results prove that ornithine and citrulline are precursors of arginine in Neurospora. It is also possible to deduce the sequence of reactions: In the first place, it is clear that arginine is the end-product of the reaction chain. This follows from the observation that arginine is the only amino acid which satisfies the requirements of all the mutants, and is in agreement with the fact that arginine is the only one of the three which occurs generally in proteins. It is possible to write three sequences by which ornithine and citrulline might be converted to arginine:

(1). C → O → A
(2). O → A ← C
(3). O → C → A

On the first hypothesis it would be impossible to obtain a strain which could utilize citrulline but not ornithine. Two such strains have been found, however. According to the second scheme, citrulline and ornithine are converted to arginine over independent routes. Accord-

ingly, it should be possible to obtain strains which can utilize ornithine, but not citrulline. No such strains have been found. The third sequence is the only one which is capable of explaining all the data. According to it, four of the mutants are unable to synthesize ornithine from sugar and ammonia, but can convert it to citrulline and arginine. Two mutants are blocked in the conversion of ornithine to citrulline, while one is unable to convert citrulline to arginine (Fig. 1). It is of interest to

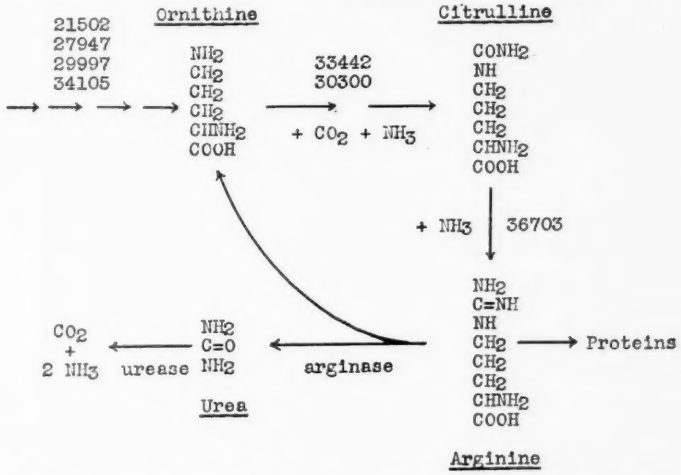

FIG. 1. The ornithine cycle in *Neurospora*. The numbers designate the genes controlling the various steps.

note in connection with these reactions that Neurospora contains the enzyme arginase, which catalyzes the hydrolysis of arginine into one molecule of ornithine and one molecule of urea. The process outlined above is therefore cyclical, one molecule of urea being produced every time the cycle is repeated. This series of reactions, known as the ornithine cycle, was first suggested by Krebs and Henseleit (1932) in their well-known study of urea synthesis in the mammalian liver. Unlike the mammal, however, Neurospora does not excrete the urea so produced, but breaks it down into carbon dioxide and ammonia by means of the enzyme urease. By studies

based on genetic material, it has thus been possible to show that such a typically animal function as urea synthesis also occurs in the plant kingdom, and by the same series of reactions.

A second example of this method of attack has to do with the synthesis of the amino acid tryptophane. Thirty-three mutants of Neurospora have been obtained which are unable to grow in the absence of added tryptophane. Genetic evidence shows that at least two different loci are involved. By a study of the specific growth requirements of these strains Tatum, Bonner and Beadle (1944) have been able to show that they fall into two gen-

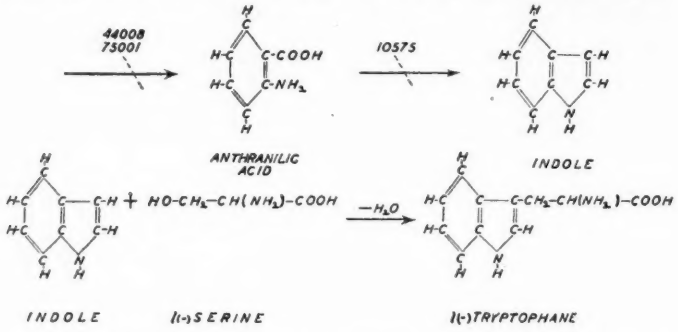

FIG. 2. Tryptophane synthesis in *Neurospora*. The numbers designate the genes controlling the various steps.

eral classes: those which can utilize either anthranilic acid or indole in place of tryptophane, and those which can utilize indole but not anthranilic acid. It is evident that both anthranilic acid and indole can serve as tryptophane precursors for Neurospora, and by the same line of reasoning as was used in the previous example, anthranilic acid precedes indole in the reaction chain (Fig. 2). Further work on the chemistry of these reactions showed that in making a molecule of tryptophane, indole combines with the amino acid serine (Tatum and Bonner, 1944). These findings have for the first time established a mechanism of tryptophane synthesis in living organisms.

The kind of approach used in the analysis of the

arginine and tryptophane series of mutants is not practical in many cases, since, in the absence of previous clues, the range of possible precursors of a complex organic molecule is very large. In such cases, the methods of cross-feeding and chemical isolation have been used. If a chain of reactions is blocked at some point the intermediates behind the block will tend to pile up, like water behind a dam. These intermediates may be excreted unchanged, or they may undergo secondary changes of one kind or another. The classical case of accumulation of an intermediate as the result of a gene mutation was mentioned previously—namely, alcaptonuria, in which homogentisic acid is excreted. In alcaptonuria the presence of an abnormal constituent in the urine was indicated by the gradual blackening of the urine when exposed to air. A number of similar cases have occurred among the Neurospora mutants. For example, the mutant which is unable to convert anthranilic acid to indole was found by Tatum, Bonner and Beadle (1944) to excrete anthranilic acid into the medium in sufficient quantities to produce fluorescence. Final proof was obtained by the isolation and identification of anthranilic acid in media on which the mutant had grown. Another interesting case is that of a mutant which requires supplementary adenine for growth. This strain differs visibly from wild type in that it gradually accumulates a bright purple pigment in its mycelia. The pigment has been obtained in purified form by Mitchell (unpublished). It appears to be a highly polymerized form of a purine-like molecule. In agreement with the hypothesis that the pigment is the product of a blocked reaction, it has been found that the purple color is epistatic to albino.

The isolation of accumulated intermediates is not limited, however, to substances which produce a visible change in the mycelium or medium. If two or more mutant strains are available, each of which is blocked at a different point in the same synthesis, it is often found that one strain produces a substance which can be utilized by another strain in place of the end product. Thus, the

anthranilic acid produced by one tryptophane mutant can be used by others in which the point of block occurs somewhere behind anthranilic acid. Of the four known choline-requiring strains of Neurospora, two represent mutations of different genes. At the present writing, tests of a number of possible choline precursors have shown no differences in the specificity of the two strains, although one of them responds to much lower concentrations of choline than does the other. In a cross-feeding experiment, however, it was found that one of the mutants excretes a substance into the medium which can be utilized by the other mutant (Horowitz, unpublished). The mutant is now being grown on a large scale, in five-gallon carboys, from which it is hoped the intermediate can be isolated.

From the results of the Neurospora work so far, it is possible to draw two main conclusions. First, the synthesis of the essential chemical constituents of living matter is under genic control. It seems probable that the requirements of higher animals for dietary supplements of vitamins and amino acids are the result of gene mutations which have occurred in the evolution of species. Although it is going beyond our present information to suggest a mechanism of this control, it appears that the primary action of the gene has to do with the synthesis of the enzymes which direct the chemical activities of the cell. This hypothesis seems capable of explaining not only the known mutations in Neurospora, but many other genetic data as well. We are indebted to Dr. Sewall Wright (1941) for much of the development of this concept, both in its experimental and theoretical aspects.

The second conclusion we may draw is that a one-to-one correspondence exists between gene and chemical reaction. It follows that the number of genes concerned in the synthesis of a single substance approaches the number of chemical steps involved. Studies of the Neurospora mutants has made it possible in a number of cases to assign definite reactions to individual members of a series of non-allelic genes.

In closing this review, it may be said that much still remains to be done in the investigation of these mutants. It seems to us that the analysis of metabolism by means of single-gene mutants is a method comparable in scope to the isotope method of tracing the history of molecules in the organism, while on the genetical side, the reduction of gene effects to simple chemical reactions would seem to be the first step in the direction of further analysis of gene action.

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THE DEPENDENCE OF THE PHYSIOLOGICAL ACTION OF A GENE ON A PRIMER AND THE RELATION OF PRIMER TO GENE[1]

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THE determination of each hereditary difference thus far examined in certain varieties of the ciliated protozoan *Paramecium aurelia* involves a cytoplasmic factor. In the cases analyzed further, the cytoplasmic factor is not the sole determinant, but depends in a peculiar way upon a gene. In order to understand the action of such a gene as a physiological agent, the interrelations between gene and cytoplasmic factor need to be examined in detail. The present paper, after briefly reviewing what has already been published (Sonneborn, 1943) on these interrelations in the most fully studied case, deals with a series of new experiments which carry the analysis further.

I. REVIEW

The interrelations between gene and cytoplasmic factor have been most fully studied in the case of the determination of the alternative characters "killer" and "sensitive" in variety 4 of *P. aurelia.* One race of this variety makes the fluid in which it lives poisonous to nearly all other races of Paramecium. This race is therefore a "killer." It might as well be called "resistant," for it is also resistant to its own poison. The races affected by this poison may be called either "sensitives" or "non-killers," for "sensitives" are never "killers." Indeed, clones have these four characters in just two com-

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binations: any clone is either a resistant killer or a sensitive non-killer.

The alternative characters, killer and non-killer, are determined by a pair of allelic genes, K and k, and a cytoplasmic factor, which may be called kappa. Clones are killers only when both the dominant gene K and the cytoplasmic factor kappa are present; they are non-killers when kappa is absent, regardless of genic constitution.

The interrelations of K and kappa were investigated by studying separately the effects of each of these two factors, when removed from the other one. The effects of kappa in the absence of K were observed by obtaining self-fertilization (autogamy) within heterozygous killers (Kk plus kappa). In the resulting homozygous recessives (kk), the K genes in the nuclei have been replaced by k; but the cytoplasm is, at least at first, the same as before and so contains kappa. These recessives manifest the killer character for several fissions, then become and permanently remain non-killers. The significance of the change of phenotype in relation to kappa is indicated when gene K is reintroduced into the recessives by mating them to homozygous dominants[3] (KK). When only a few fissions intervene between the origin of the recessives from their heterozygous parents and the subsequent mating to the dominants, the reintroduction of gene K results in preventing the loss of the killer character. When more fissions intervene, the reintroduction of the killer gene is without effect: a non-killer clone develops. The gene K thus serves as a detector for the presence of kappa, and the results indicate that kappa persists in the recessives for a few fissions and then permanently disappears. Persistence of kappa in the recessives is correlated with the persistence of the killer character for a short time, indicating that kappa alone can determine the killer character in the absence of K from the nuclei. The

[3] In published experiments, the dominants were killers. Subsequently the experiment has been repeated with the same results using KK non-killers lacking kappa.

eventual loss of kappa, however, shows that it is not independently self-multiplying in the absence of K. Gene K thus controls the maintenance (and consequent increase in amount) of kappa during reproduction by fission. This is its only known function.

The fact that after gene K has been lacking from the nuclei for several fissions its reintroduction fails to restore the killer character to recessives containing cytoplasm derived exclusively from the killer race indicates not only that kappa has disappeared, but that gene K is unable to initiate the production of kappa. This is also clearly shown in much the same way by transferring K but not kappa to a pure non-killer race (kk) that possesses neither gene K nor cytoplasm of the killer type. The method is simply to cross killers (KK plus kappa) to non-killers (kk). At conjugation, each conjugant supplies the other with a gamete nucleus, but cytoplasm is normally not exchanged. Consequently, gene K is introduced into the non-killer, leaving kappa behind. The resulting heterozygote and all its vegetative and inbred sexual progeny remain permanently non-killers. Hence, both in heterozygotic and in homozygotic condition, K can not initiate the production of kappa and therefore can not determine the killer character. Nor can any other gene from the killer race initiate the production of kappa, because all of them, like K, are introduced into the non-killer at conjugation and are brought into homozygous condition in the F2.

To the non-killers containing K, obtained as just set forth, kappa may be added by conjugation with killers under certain conditions in which cytoplasm is exchanged between the mates (see experiment 1, p. 322, for details). The introduction of cytoplasm from the killer into the non-killer mate results in the production of exclusively killer progeny thereafter in both vegetative and sexual reproduction. Hence, kappa is maintained and increased in the presence of K when some kappa is present to start with.

The knowledge of kappa and K up to this point may be summarized as follows. Kappa is (1) absent from the migratory gamete nucleus; (2) present in the cytoplasm of killers at the time of fertilization; (3) essential for the development of the killer phenotype; (4) physiologically active even in the absence of gene K from the nuclei; (5) dependent upon gene K for its increase during growth and reproduction and so unable to persist after gene K is replaced by its allele k; (6) not initially producible by gene K or any other gene in the killer race. Dominant gene K (1) is unable to initiate kappa production, but (2) controls increase of kappa when kappa is already present.

II. Further Analysis of Interrelations between K and Kappa

The facts set forth in the preceding review indicate that kappa acts something like a primer in a pump: some kappa is put in and more comes out. The gene K seems to be like a pump that will not work at all without being primed: in the absence of the primer, no activity of the gene is detected. K thus appears to be a gene whose observable physiological activity—the production of kappa—is dependent upon a primer, kappa itself. This characterization of kappa as a primer of gene K is, however, merely a superficial description of a phenomenon that requires further analysis. Possibly the direct action of gene K is entirely independent of kappa. K might produce a product which has no observed effect in the absence of kappa, but which interacts with kappa to form more kappa when kappa is present. Still other and more complex interpretations are possible: for example, kappa might represent two cytoplasmic components, one independently self-multiplying and the other a primer of gene K, the two components interacting to determine the killer character. These considerations will suffice to show that the detailed relations of kappa to gene K remain to be discovered and that these relations must be known before the physiological activity of gene K can be understood.

In an effort to acquire some of the needed information, the experiments now to be set forth were performed.

Experiment 1. The relation between diverse periods of paroral union and the transfer of kappa during conjugation.

Soon after fertilization has been accomplished, conjugating mates normally separate. As a rule separation takes place last at a very small region near the cytostomes, the paroral cones, across which the migrating gamete nuclei pass during fertilization. At room temperature the great majority of conjugating pairs in all crosses (and all, or practically all, in crosses involving certain races) break apart at the paroral cones within $3\frac{1}{2}$ minutes of the moment when the process of separation first appears in the anterior region. In certain crosses, however, some pairs remain united at the paroral cones for longer periods, in the extreme case remaining permanently united and eventually fusing to form a double animal. In the latter and exceptional case, cytoplasmic mixture is complete; normally, however, no cytoplasm is exchanged at conjugation. As will appear, intermediate conditions exist and, further, the amount of cytoplasmic exchange is correlated with the extent of the period of paroral union. This permits the selection and study of conjugants into which varying amounts of the cytoplasmic factor kappa have been introduced.

Crosses were made between killers (KK plus kappa) and non-killers homozygous for the killer gene (KK but lacking kappa). If cytoplasm containing kappa passes from the killer to the non-killer mate, the latter should yield killer instead of non-killer progeny (see p. 319). This test was therefore applied to pairs of conjugants which had been observed to remain united at the paroral cones for diverse periods after separating at the anterior ends.

When the period of paroral union lasted $3\frac{1}{2}$ minutes or less, the non-killer conjugant never yielded any killer progeny. Hence, under these conditions, no kappa (or

effectively none) passed from the killer to the non-killer conjugant. At the other extreme, when the period of paroral union lasted more than about 30 minutes, the non-killer conjugant always promptly produced a clone of killers (as in the case mentioned on p. 319). Hence, under these conditions, kappa invariably passes across from one mate to the other. Between these two extremes, when the period of paroral union lasts from 3½ to 30 minutes, a variety of results is obtained. Some of the non-killer conjugants yield exclusively non-killer progeny, some quickly develop into killer clones, and some yield clones of a type previously not encountered. Clones of this new type remain non-killers, but, unlike non-killer clones previously studied, they are capable of producing killer progeny under certain conditions to be specified later. The latter fact suggests and experiments 2 and 3 demonstrate that this new type of non-killer clone contains kappa. Further, the data indicate that the amount of kappa transferred to conjugants that produce clones of this type is less than the amount transferred to conjugants that produce killer clones, for this new type of non-killer clone arose only when the period of paroral union was intermediate between the period (less than 3½ minutes) in which no kappa is transferred and the period (more than 30 minutes) in which enough is always transferred to render the resulting clone a killer. There was, however, much overlapping of results: in some pairs with paroral unions of only 6 minutes, the non-killer conjugant produced a clone of killers; in other pairs with paroral unions as long as 9 minutes, the non-killer conjugant produced a clone of non-killers lacking kappa. Hence the duration of paroral union is only a crude index of the amount of cytoplasm and kappa transferred.

Experiment 2. Comparison of the new type of non-killer clone with its progeny produced by self-fertilization (autogamy). Proof that the new type of non-killer clone contains kappa and every other factor or substance required for the killer character.

Autogamy, or fertilization within single unmated cells, is readily induced at any time after clones are 10 to 12 fissions old. When a clone of the new type of non-killers is divided into two parts and one part is induced to undergo autogamy, while the other part is prevented from undergoing autogamy, the following results are obtained. The part that has been prevented from undergoing autogamy, but has continued to reproduce by fissions, retains the non-killer character. The part that has been permitted to undergo autogamy transforms promptly into killers. If many individuals undergoing autogamy are isolated and separately cultivated, as a rule every one of them produces a clone of killers. (The preceding results are those typically obtained when the non-killer clones are young; exceptions, further details and results with old clones are given below in experiment 4.)

Experiments 2 shows clearly that non-killer clones of the new type contain kappa and every other factor or substance required for the determination of the killer character because they are transformed into killers by means of a process (autogamy) which occurs within single cells and so excludes the introduction of material from another organism. How, then, do they differ from killers? The difference is certainly not due to a difference in genes, for the following reasons. First, the parents from which these non-killers were derived (experiment 1) were both already homozygous for the killer gene K. Second, both parents were also homozygous for all their other genes, though not necessarily for the same genes, because just a few days before the cross was made they had been through autogamy, which renders them homozygous for every gene (Sonneborn, 1942). Consequently they could yield only one genotype when crossed. The non-killers with kappa that were obtained from the cross must therefore have been genically identical with the killers obtained at the same time from the same cross. Third, when the non-killers with kappa underwent autog-

amy, as a rule 100 per cent. of the self-fertilized cells produced killer clones. Genic recombinations do not give that sort of result.

As non-killers that contain kappa differ from killers neither in their genes nor in the kinds of other factors or substances present, it seems that the only differences that could distinguish them are the amount of kappa they contain or the form in which it exists. As experiment 1 shows that non-killers with kappa arise only when *relatively small amounts* of kappa are introduced into KK non-killer conjugants, the failure to develop the killer character seems to be the result of this fact. If the kappa is in an inactive form in these clones, this, too, would probably be a consequence of the initial deficiency in quantity of kappa. It thus appears that the phenotype depends not only on the kinds of determiners present (K and kappa), but also on the quantity of one of them (kappa). It is remarkable that the quantity of kappa present at the time of fertilization should determine the phenotype for long periods of reproduction by fissions. To this we return later.

Experiment 3. Second proof that the new type of non-killer clone contains kappa. Cross between KK non-killers of the new type and KK non-killers known to lack kappa.

The existence of kappa in the new type of non-killers may be tested further by ascertaining whether they are capable of transferring kappa to their mates at conjugation. Crosses were therefore made between a clone of the new type of non-killers and a clone of KK non-killers known not to contain kappa (*i.e.*, one incapable of yielding killer progeny when inbred). In most of the pairs, those that separated rapidly after fertilization, one exconjugant produced a clone of killers and the other exconjugant a clone of non-killers that was incapable of yielding killer progeny at autogamy. The new type of non-killer thus transforms into a killer at conjugation as well as at autogamy. In some of the pairs, those that

separated slowly after fertilization, both exconjugants produced clones of killers. Clearly non-killers of the new type had transferred to their non-killer mates the kappa required to convert them into killers. In a few pairs, some of those with intermediate periods of paroral union, one exconjugant gave a clone of killers and the other a clone of non-killers of the new type (*i.e.*, it transformed into killers whenever autogamy occurred). In other words, in these pairs also both exconjugants produced clones containing kappa and, therefore, kappa must have been transferred from one mate to the other. Hence the new type of non-killer clone contains kappa, for it not only can transform into a killer clone at fertilization, but also can transfer kappa to its mate at conjugation, thus rendering a non-killer conjugant that lacked kappa capable of producing killer progeny. This experiment also shows that the kappa in non-killers must be in the cytoplasm at the time of fertilization; otherwise it could not pass into the mate.

Experiment 4. Comparison of the effects of autogamy at different ages and in different lines of descent in clones of non-killers that contain kappa. Proof that kappa is unequally divided at fission. Evidence that this is the basis of the difference in effects of autogamy in different individuals of a clone.

Clones of non-killers that contain kappa were obtained in the way explained in experiment 1. Each of the first two, four or eight animals produced at the first fissions was isolated and separately cultivated by transfers of a single animal of each line of descent daily. The animals (usually 31 in number) left over at the time of each daily isolation were grown for less than one more day until 250 to 500 animals were present and these cultures were then tested to ascertain whether the isolation lines were killers or non-killers. Periodically, some animals from these cultures were allowed to go into autogamy, the autogamous animals were isolated and grown, and the resulting cultures were tested to ascertain whether the

isolation line produced killer or non-killer progeny at autogamy. The results were different even in different lines of descent of the same clone. There were three types of lines of descent, as set forth in the following paragraphs.

(1) Very rarely, a line of descent contained no kappa at any stage of its history. Only three such lines have been found. Two of these included all the progeny of one of the first two cells produced at the first fission of an exconjugant; one included all the progeny of one of the first four cells produced at the second fission of an exconjugant. At every test these lines were non-killers and they never produced any killer progeny at autogamy. Thus kappa can be absent from one of the first two or four cells and be maintained in the other one or three cells and their progeny. Kappa is therefore occasionally divided unequally among the very first cells produced in the clone.

(2) Many lines of descent maintained kappa for 18 to 88 successive cell divisions and then lost it. These lines also remained permanently non-killers. Some of them were followed as daily isolation cultures until they died. (Clones of *P. aurelia* eventually die in the absence of fertilization (Sonneborn, 1935); in variety 4 death occurs in less than 200 fissions, usually much less.) In lines of this type, the results of autogamy differed at different stages of the life history. At any time from the origin of the clone up to a certain stage, as a rule 100 per cent. of the animals that went into autogamy yielded clones of killers. There then followed a period in which autogamy yielded lower percentages of killer clones and finally none at all. Once the latter result was obtained, autogamy never yielded killer clones at any later time. During the transition period, the cells that went through autogamy and did not transform into killers of course remained non-killers. Of the non-killers produced, the majority were incapable of giving rise to killers at any subsequent autogamy, but the remainder did yield killers at the next

autogamy. The latter, therefore, were like the parent clone: they were non-killers that nevertheless contained kappa. However, when the stage at which no killers were producible at autogamy was reached, all the non-killers were of the permanent type that did not yield killers at any later autogamy.

The existence of a transition period, in which less than 100 per cent. of the cells undergoing autogamy produce killer clones, indicates both that the amount of kappa per cell decreases gradually before it disappears and also that the percentage is a measure of the amount of kappa in the parent line. As this percentage did not always follow a steady downward course, a higher percentage of killers sometimes resulting from a later autogamy, the amount of kappa in a line of descent seems to be capable both of decreasing and of increasing during the passage of fissions, indicating again that kappa is unequally divided at fission.

The final period, in which autogamy yields only non-killer clones lacking kappa, begins in some lines as early as the 18th fission, in others as late as the 88th fission. As the capacity to produce killer progeny at autogamy is the test for the presence of kappa in the parent non-killer line, kappa disappears from these lines after having been maintained for from 18 to 88 fissions. This fact is in striking contrast to previous observations demonstrating the permanent maintenance of kappa in the presence of gene K. But previously observations were made on KK clones obtained from conjugants into which enough kappa was introduced to enable them to produce killer progeny quickly during vegetative reproduction; under such conditions, kappa is maintained indefinitely under the action of gene K. The present observations were made on KK clones obtained from conjugants into which too little kappa was introduced to enable them to produce killer progeny during vegetative reproduction; in such cases, kappa may be maintained for enormously long or for shorter periods and then disappear entirely.

(3) In addition to the two preceding types of lines, there was a third which was the most common of all. In lines of this third type kappa *never* disappeared in the course of vegetative reproduction. In some of them autogamy invariably resulted in transformation into killers: at every stage of life, every cell that went into autogamy gave rise to a clone of killers. In others, however, there occurred periods in the life history in which autogamy yielded less than 100 per cent. killer clones. At these periods the results of autogamy were the same as in the lines that lost kappa, during the period just before its loss; but in lines of this type, kappa did not disappear, and later the line again yielded 100 per cent. killer clones at autogamy. It would seem, therefore, that in these lines also the amount of kappa per cell may fluctuate from fission to fission.

During vegetative reproduction the character of lines that never lost kappa was, at least for long periods, identical with the character of the others: they remained phenotypically non-killers. Eventually, however, they seemed to change into killers, usually after 60 to 80 fissions, though occasionally after as little as 30 fissions or more than 100 fissions. Whether the observed transformation into killers actually occurred during purely vegetative reproduction is open to question for the following reason. As has been mentioned, the tests for phenotype were made, not on the daily isolation lines themselves, but on cultures grown from the animals left over at the time of the daily transfers. A technical difficulty arises from the fact that as clones grow older they become more and more apt to undergo autogamy in small mass cultures of this type, though in the isolation lines autogamy does not occur so readily and can be entirely avoided by selection (Sonneborn, 1938). As has been shown, the lines transform into killers at autogamy. Hence, if the cultures underwent autogamy before or during the test, this would make it impossible to ascertain the phenotype of the parent isolation line. The technical difficulties involved in

getting a clear-cut answer to the question at issue are very great; until these difficulties are overcome, the question whether non-killers ever transform into killers during vegetative reproduction must be left open. On the other hand, it is absolutely clear that the lines under consideration retained kappa permanently: they were always capable of yielding killers at autogamy.

The three preceding diverse types of lines in the clones of non-killers that contained kappa occurred side by side within the same clone. They differ basically in only one respect: some lines lose kappa at once, some much later, others not at all. The first two types of lines described lose kappa, one immediately and the other later; the third type retains kappa permanently.

One feature common to two of the three types of lines now requires consideration. How does it happen that when the amount of kappa per cell is low in the parent non-killer line, some cells of the line yield killer clones after autogamy while at the same time some cells yield non-killer clones containing kappa and others yield non-killer clones lacking kappa? The following considerations suggest the answer. As pointed out (p. 326), autogamy was induced in the progeny of the animals left over after the daily isolation has been made (*i.e.*, among a group of about 500 animals). As shown above, kappa is unequally divided at fission and but little kappa was present in the parent line during the period under consideration. Doubtless among the many animals of any group in which autogamy was induced at that time, some had more kappa, some had less and some had none. Probably the three results of autogamy were due to its occurrence in those three kinds of animals: those with more kappa yielded killer clones; those with less, non-killer clones containing kappa; those with none, non-killer clones lacking kappa. Thus the action of autogamy could be uniform and the differences in results could be due simply to differences with respect to the kappa content of the cells in which autogamy takes place.

Certain further facts confirm this interpretation. A line of non-killers had maintained kappa for 33 fissions. At this time, instead of continuing the daily isolation line with one animal, two animals were isolated and separately cultivated. One of these yielded progeny in which kappa could never again be detected, though the first test was made on its immediate offspring; the sister cell yielded progeny which retained kappa permanently. This demonstrates how, at a critical stage, a group of only 32 animals descended from one during five fissions can include some that have and some that have not lost kappa. Had autogamy been induced in the 31 animals left over after the isolation following the 33rd fission, it would necessarily have taken place in animals differing in precisely the way postulated to account for the diversity in its effects. Thus kappa is unquestionably lost from some animals during vegetative reproduction, and autogamy in these can not restore it. Further, at times when some animals of the clone lose kappa, there must also be some animals containing intermediate amounts of kappa; and only at such times does autogamy in non-killers yield non-killers containing kappa. There can therefore be little doubt that this intermediate consequence of autogamy is due to its occurrence in animals containing intermediate amounts of kappa.

III. Summary and Discussion

(1) *Summary of results.* The preceding experiments have brought to light a theoretically important fact: the existence of clones of *non-killers* that contain both gene (K) and cytoplasmic factor (kappa) hitherto thought to be sufficient for the determination of the *killer* phenotype. In previous experience, non-killer clones always lacked either kappa or both kappa and gene K. The experiments also supplied the following information about the new type of non-killer clone. First, it is genically identical with killer clones. Second, it lacks no kind of cytoplasmic factor required for the killer phenotype. Third,

it originates when the amount of kappa in a cell is low at the time of fertilization. Fourth, the kappa it contains is unequally divided at fission. This results in the loss of kappa from certain lines of descent within a clone after it has been maintained for from one to more than 88 fissions; but it also results in the permanent maintenance of kappa in most lines of descent. The question whether this unequal division of kappa at fission ever results in the transformation of non-killers into killers during vegetative reproduction is left open; but it is certain that such transformation, if it occurs, does not usually take place until after 60 to 80 successive fissions. Fifth, fertilization in the new type of non-killer clone results in the production of three types of clones—killers, non-killers with kappa, non-killers lacking kappa—and these three results seem to be determined by whether the cell undergoing fertilization has at that time more, less or no kappa respectively.

(2) *Problems.* The preceding results present four problems that must be solved in order to gain further understanding of the interrelations of K and kappa and their action in the determination of the phenotype. First, how is the difference between killers and non-killers that contain both K and kappa determined? Second, how can the unequal division of kappa at fission be accounted for? Third, how does fertilization accomplish the transformation of non-killers that contain kappa into killers? Fourth, how does it happen that the amount of kappa in a cell at the time of fertilization determines its character permanently or at least for very long periods?

(3) *Interpretations.* The experiments reported above do not provide answers to the preceding questions. However, they do provide a basis for discussing what seem to be the possible answers.

The most obvious answer that might be given is that the difference between killers and non-killers that contain kappa is due simply to a difference in the *amount* of kappa they contain. The observations in experiment 1

show that when KK non-killers receive much kappa they become killers and that non-killers containing kappa arise only when they receive less kappa. Any hypothesis must allow for this fact and must to that extent include a quantitative aspect. On the present hypothesis, the unequal division of kappa at fission would follow from its cytoplasmic localization and the lack of any precise mechanism for equal division of cytoplasm at fission. The transformation of non-killers into killers at fertilization would necessarily mean that some process or processes involved in or associated with fertilization increase the amount of kappa, though the mechanism by which this happens does not appear to be deducible from the hypothesis. To explain the increase of kappa at fertilization, additional assumptions would probably have to be made. The long or permanent persistence of the non-killer character during vegetative reproduction would imply that the increase of kappa keeps close pace with the increase in cell size and number; in other words, kappa would have to be approximately doubled during each cycle from fission to fission.

The only apparent alternative to the simple quantitative interpretation just outlined is to assume that the kappa is in an inactive form in non-killers, an active form in killers. To distinguish this from the simple quantitative hypothesis and at the same time to reconcile it with the fact that the kappa introduced into a KK cell is inactive only when small amounts are introduced, it would have to be assumed further that KK cells contain a limited amount of something that inactivates kappa without destroying it and without preventing its increase during vegetative reproduction. The inactivated form would then have to be activated in some unknown way at the time of fertilization, but never (or not for long periods) during vegetative reproduction. The inactivation hypothesis in this general form is thus more complex and therefore *a priori* less acceptable than the simple quantitative hypothesis.

On the other hand, certain general considerations, which need not be recounted here, suggested that the K genes in the macronucleus might be the inactivators of kappa, and this concrete form of the inactivation hypothesis appears to agree with and explain the results in the experiments reported in this paper better than the simple quantitative hypothesis. Unlike the latter, it explains the remarkable transformations of character at fertilization without additional assumptions. Further, it leads to predictions seemingly incompatible with the alternative hypothesis, predictions that have been confirmed by experiment. The following paragraphs attempt to set forth briefly these aspects of the special form of the inactivation hypothesis, which is perhaps better designated as the hypothesis of the combination of kappa with gene K in the macronucleus.

According to this hypothesis, the macronuclear K genes are capable of combining with kappa and thus of withdrawing it from the cytoplasm into the macronucleus. When only little kappa is available in the cytoplasm, all of it may combine with and be bound to the K genes, leaving none in the cytoplasm; when much kappa is available in the cytoplasm, the macronuclear K genes become saturated with it and leave some in the cytoplasm. These two conditions correspond to the non-killers that contain kappa and the killers, respectively. The inactivation of kappa is thus a consequence of its confinement to the macronucleus; it is active (*i.e.*, produces the killer character) only when it is also present in the cytoplasm.

As mentioned above, this hypothesis has the merit of explaining, without resorting to additional assumptions, the remarkable changes of character brought about at the time of fertilization in KK non-killers that contain kappa. The explanation is based only on the known structure and origin of the macronucleus. The adult macronucleus is known (Sonneborn, 1940) to be a compound structure consisting of at least 20 to 40 units each of which contains at least one full diploid set of genes.

This structure disintegrates at the time of fertilization and so would be expected to release into the cytoplasm whatever kappa it might contain. Concurrently two new macronuclei develop from simple diploid micronuclei; their development consists of enormous growth, accompanied by increase in the number of component units from one to the full number characteristic of the adult macronucleus. There are thus at the start only a few K genes to draw upon the kappa that had been released into the cytoplasm by the many K genes of the disintegrated old macronucleus. If many or all of the K genes of the old macronucleus had been combined with kappa, there would thus be available for the few K genes of the young new macronuclei far more kappa than they could combine with; consequently some would be left over for the cytoplasm and this would render the resulting clone a killer. If but few of the K genes in the disintegrating macronucleus had kappa, there would be only enough, or less than enough, kappa to combine with the K genes of the new macronucleus, leaving none in the cytoplasm; consequently the resulting clone would be a non-killer with kappa. Of course, if there were no kappa in the old macronucleus, there would be none in the resulting clone. The observed three kinds of results of fertilization and their relation to the amount of kappa in the cell at the time of fertilization (experiment 4) are thus explained by and are in complete agreement with this hypothesis.

The hypothesis explains equally well the observed unequal division of kappa in clones of non-killers. As mentioned in the preceding paragraph, when the amount of available kappa in the cytoplasm at the time of fertilization is very small, there would not be enough to saturate all the K genes in the two new developing macronuclei. One new macronucleus might get some kappa, the other might get none; this would result in the segregation of kappa at the first cell division, a condition that has been twice observed (experiment 4). Or the amount present

might be so small that before it gets into the new macronuclei the latter have already begun to develop their compound structure, so that some units of the developing macronuclei combine with kappa and there is none left for the other units to combine with. The macronucleus divides amitotically as a whole, but the component units must undergo a sort of mitotic division except for the fact that the arrangement of the units is such as to result usually in the passage of both products of division of a unit to the same instead of to different daughter macronuclei. Consequently, if some units contain kappa and others lack it, in the course of successive fissions some entire macronuclei lacking kappa and also some that are completely saturated with kappa must arise. Here, then, is a mechanism which would assure the unequal division of kappa at fission whenever the macronucleus is not saturated with it.

The hypothesis also explains the persistence during vegetative reproduction of the non-killer character determined at the time of fertilization by the small amount of kappa present at that time. In the absence of any mechanism during vegetative reproduction by which kappa could be transferred from the macronucleus (where it is bound to gene K) to the cytoplasm (where it would have to go in order to bring about transformation of a non-killer into a killer), the unequal distribution of kappa at fission could result in only a limited range of variation in kappa: the macronucleus could become completely saturated, or, at the other extreme, kappa could be lost completely. In either case, the clone would remain a non-killer.

Finally, the hypothesis is also in agreement with the transfer of kappa from non-killers to their mates during conjugation, for the macronucleus disintegrates just prior to the exchange of gamete nuclei and so the kappa bound to K genes in the old macronucleus could be released into the cytoplasm in time to permit its passage into the mate in pairs of conjugants experiencing prolonged paroral union.

It might, however, be objected that, if the K genes of the macronucleus are combined with kappa, the K genes of the micronucleus should also be combined with kappa. If this were the case, kappa would regularly be transferred at conjugation whenever killers mate with non-killers, for the gamete nuclei are derived directly from the micronuclei. This clearly does not happen (see section I, Review); otherwise the existence of kappa as a cytoplasmic factor could never have been discovered. The micronuclear K genes are therefore not combined with kappa. This assumed distinction between the behavior of K genes in the two types of nuclei is, however, not as incredible as at first appears, for it is well known that micronuclear genes are in general different from macronuclear genes: much evidence indicates that the former are physiologically inactive and non-essential, while the latter are physiologically active and essential.

The hypothesis of the combination of kappa with the macronuclear K genes thus agrees with and explains the observations and experimental results. What is needed, however, is an experimental test involving diverse predictions on the two alternative hypotheses. The following test seems to fulfill this requirement. The transformation of non-killers containing kappa into killers at fertilization is, on the quantitative hypothesis, due to increase of kappa by some process involved in fertilization; but on the hypothesis of combination of kappa with K, it is due to the release into the cytoplasm by the disintegrating old compound macronucleus of more kappa than can combine with the K genes of the simple newly arising macronuclei. On the latter hypothesis, if the number of newly arising macronuclei could be greatly increased, this great increase in the number of functional K genes would remove more kappa from the cytoplasm. In the extreme case, this would result in the production of a KK non-killer from a KK killer at fertilization, a result contrary to the quantitative hypothesis.

This experiment has been performed and the results

agree with the expectations on the combination hypothesis: the amount of active, cytoplasmic kappa was reduced by increasing the number of new macronuclei formed at the time of fertilization. The full details of this experiment and of others along the same lines will be reported at another time. Here it need only be said that it was possible, in the way indicated, to obtain from pure killer races non-killers containing kappa and ultimately pure non-killers entirely lacking kappa.

This initial success of the combination hypothesis makes it worth while to state briefly what the hypothesis implies concerning the gene K and its relation to the cytoplasmic factor kappa. Apparently it implies that kappa, when combined with gene K, becomes in effect a part of the gene. Further, it renders intelligible both the failure of K alone to produce kappa and the priming activity of kappa. Finally it implies that the role of K as a physiological agent is essentially to duplicate itself and whatever is combined with it and to supply the cytoplasm with a product similar to itself in two respects: (1) in being able to combine with kappa and (2) in being able when so combined to catalyse the synthesis of more kappa.

In connection with certain features of this hyopthesis, some recent papers are of particular interest. Pollister and Pollister (1943) have shown that a constituent of the chromosome, the centromere, may become detached from the chromosome and exist outside of the nucleus, as a centriole, retaining its property of self-duplication. The parallel to the behavior postulated for kappa is obvious. Emerson (1944) has recently presented evidence that certain substances (antibodies) which can combine with gene products (antigens) can also combine with the gene itself. Again the parallel to the postulated or implied behavior of kappa is obvious.

Of the two alternative hypotheses presented here, the combination hypothesis seems both to fit the observations better and to lead to more interesting further develop-

ments. At present, however, the correct interpretation is still undecided. The important things are the facts themselves and the promise they hold of leading through further experimental analysis to a better understanding of what the gene is and how it works through a primer in the determination of a character.

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CHROMOSOMAL CONTROL OF EMBRYOGENESIS IN DROSOPHILA

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ANY full analysis of development and differentiation must rest ultimately on the nature of the hereditary materials, the genes, their reproduction and their activities in the physiological economy of the cell. In spite of the work directed toward the elucidation of the former two, we are in possession of very scanty direct knowledge of the genes themselves. Like other entities of science they are better known by what they do. This and other earlier symposia attest to that. Happily, the day is past when the view that genes have something to do with cellular physiology and development had to be fought for, and genes versus the cytoplasm raised the heat both of argument and of temper. The preceding papers in this series give rather full accounts of the integral place of genes in the synthetic activities of cells and the interaction between genes and primers which place us in a very advantageous position in considering the problems of development and differentiation. It is the object of this paper to present evidence obtained by approaching these problems in a different way which, together with the above, leads to the closer merging of the disciplines of genetics, embryology and biochemistry.

In the years of research on *Drosophila melanogaster*, especially since the introduction by Muller (1927) of the x-ray technique for the production of mutation, a splendid set of materials has been accumulated for the genetical analysis of developmental processes, but it is only recently that the potentialities of certain types of these materials have begun to be appreciated. The classical method has been to infer the normal activities of a gene by study of differences in effects between alleles. This suffers from the limitations of providing only partial

information, and gives often a superficial or even erroneous picture of what a gene is really doing. Ideally, the best approach would be to inactivate completely, or to remove, one gene at a time and investigate the biochemical and developmental consequences. This has undoubtedly been accomplished in some, or perhaps in many, of the cases reported by Horovitz, Bonner, Mitchell, Tatum and Beadle. However, it is very difficult to distinguish cytologically any changes in the chromosomes of *Neurospora* which would indicate losses or rearrangements of genes. In *Drosophila,* on the other hand, the giant chromosomes of the salivary glands do enable us to distinguish cytological changes on a relatively fine scale. In this respect *Drosophila* has an advantage over *Neurospora,* in establishing the functions of parts of the chromosomes in relation to the standard genetic maps built up by Morgan, Bridges, Sturtevant and others.

The analysis of mutant types in *Drosophila* has proved that these are not always the result of gene mutation in the ordinary sense, but may represent deficiencies or duplications of greater or lesser portions of chromosomes. In some cases the mutant type is associated with more complex rearrangements in the chromosomes. Rearrangements without gross phenotypic manifestations are also known. But, as clearly foreshadowed by the classical research of Theodor Boveri (1902), most chromosomal deficiencies are lethal when homozygous. Conversely a large proportion of what have been called lethal genes have proved to be deficiencies. Lethal genes have always commanded the interest of the embryologist and have illuminated many developmental problems. Witness the work of Bonnevie, Chase, Chesley, Danforth, Dunn, Glücksohn-Shoenheimer, Grüneberg, Mohr and Wright on mammalian lethals. When a lethal is known to be a deficiency the simplest explanation of the lethal effects is that the loss involves a gene or genes concerned with some essential processes.

The large number of X-chromosome deficiencies and

translocations in *Drosophila melanogaster* and the relative ease with which they may be handled led to the choice of the X-chromosome (Fig. 1), as the starting point for the investigation of the chromosomal control of developmental processes. By appropriate crosses it is possible to obtain zygotes which lack greater or lesser parts of a given chromosome (Fig. 2). The missing part may range in size from an entire element to less than a single band of a giant chromosome. Since these deficiencies are almost always lethal to the organism, a means of evaluating the functions of a gene in normal development is

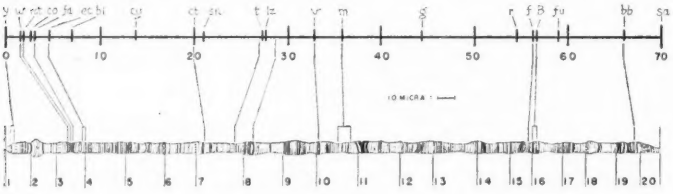

FIG. 1. Linkage and salivary gland chromosome maps of the X-chromosome of *D. melanogaster* to show the relative positions of a number of loci. Symbols of the mutants referred to in the text are: y—yellow; w—white; rst—roughest; fa—facet; sn—singed; lz—lozenge; r—rudimentary; fu—fused; sa—spindle attachment region. The numbers below the linkage map give the distances in terms of cross over units from the tip of the X. The numbers beneath the salivary gland chromosome designate the regions according to the system of Bridges. (Adapted and re-drawn after Bridges.)

made possible by the analysis of the upsets leading to the death of the zygote carrying them. The types of embryological upsets are different in the several cases studied and make it evident that a large number of chromosomal regions are concerned in embryogenesis. In all the cases considered here the lethal effects appear in the egg stage and have been determined by examining living and fixed eggs from deficiency stocks at successive times after fertilization. This is readily done, as the larva of *Drosophila* normally hatches from the egg within twenty-four hours after fertilization.

NORMAL DEVELOPMENT

The sequence of embryonic development in *Drosophila*

(Fig. 3), and the higher Diptera is briefly as follows.[1] Fusion of the pronuclei takes place in the anterior portion of the egg followed by seven or eight mitoses after which the number of pre-blastoderm, or cleavage nuclei, reaches

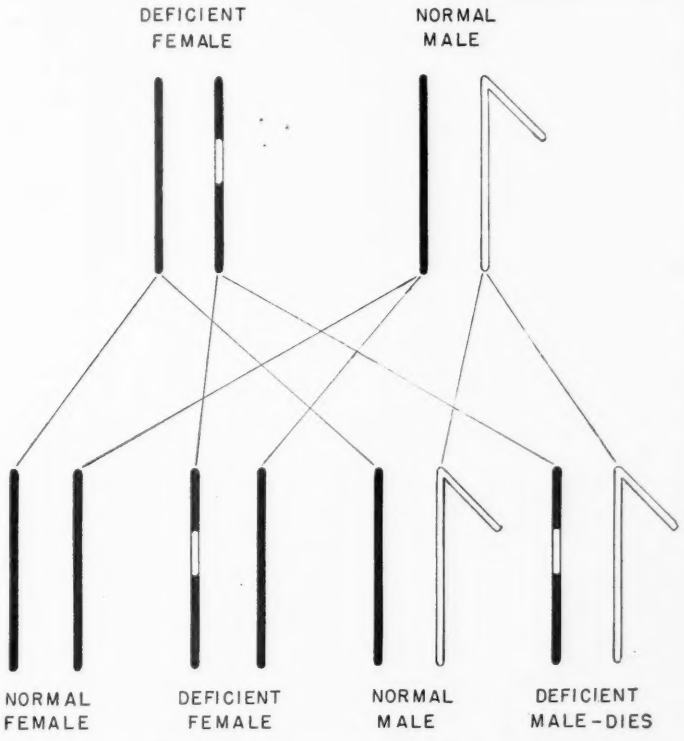

FIG. 2. The inheritance of X-chromosome deficiencies through heterozygous females. Of the four classes of zygotes only the deficient male dies at some point in development depending on the deficiency. The embryology of the others is normal.

about 256. The nuclei first become distributed more or less uniformly through the egg and are connected by cytoplasmic strands and bridges among the yolk spheres

[1] This account is based on the work of Huettner (1923, 1924), Parks (1936), Poulson (1937, 1940a, and in press), Rabinowitz (1941), and Sonneblick (1941, and in press).

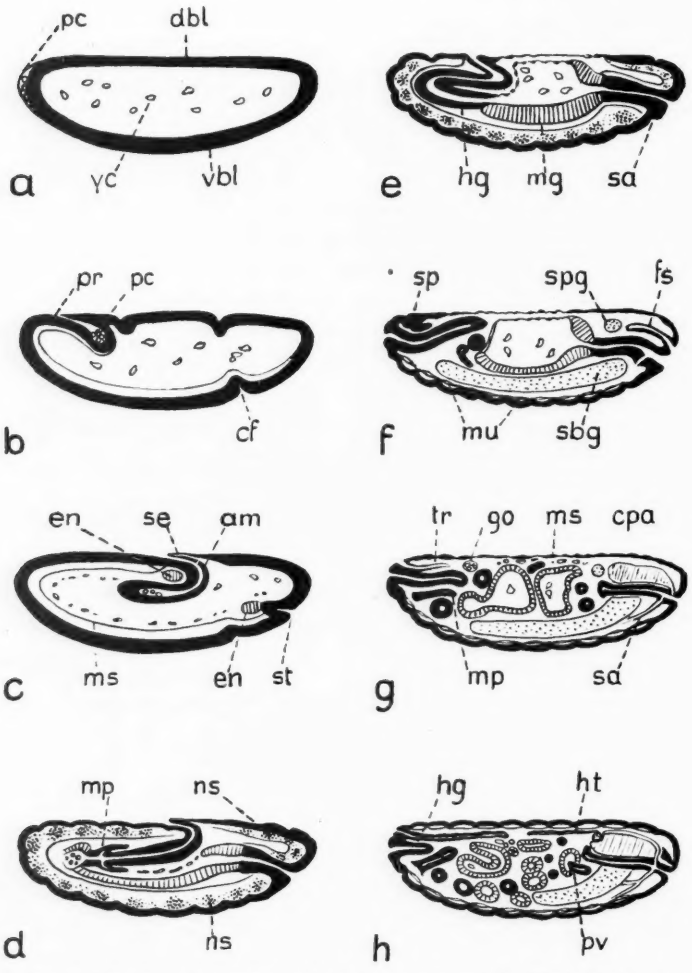

FIG. 3. Outline of morphogenesis and organogenesis in *Drosophila* from the blastoderm stage to the larva ready to hatch. Blastoderm and ectodermal derivatives shown in black, nervous system dotted; the mid-gut is cross-lined; the mesoderm unlined. a—blastoderm (2nd hr.); b—gastrulation (3rd hr.); c—extended germ band (4–6th hr.); d—later germ band showing nervous system (8th hr.); e—the contraction of the germ band (11th hr.); f—embryo following contraction (12th hr.); g—differentiation in embryo (16th hr.); h—larva before hatching (22nd hr.). Abbreviations: am—

and granules. The nuclei then begin a migration into the cortical cytoplasm of the egg. Those which reach the posterior polar plasm are differentiated almost at once to become pole cells, the future germ cells.

The nuclei which reach the cortical layer elsewhere become the blastoderm nuclei (Fig. 4a). Those which remain in the central portion of the egg become the so-called yolk or vitellophag nuclei. After several more mitoses of the blastoderm nuclei cleavage of cytoplasm between the nuclei begins and in a short time a cellular blastoderm is established surrounding the central yolk (Fig. 4d). In the third hour of development foldings in the blastoderm lead to the inturning of mid-ventral cells which constitutes gastrulation, separating outer and inner layers (Fig. 3b). Anterior and posterior portions of the latter give rise to the mid-gut and are therefore referred to as endoderm. Most of the inner layer gives rise to the mesodermal derivatives. At the posterior end of the gastrular furrow the proctodaeum is invaginated; slightly later at the anterior end, the stomodaeum. The dorsal portion of the blastoderm becomes mostly embryonic membrane. The germ band thus laid down increases in length and extends around posteriorly to the dorsal side of the egg, folding up the membranes until the proctodaeum lies just back of the head (Fig. 3c). When this extension is completed certain cells all along the ventral midline of the embryo become enlarged to form neuroblasts and move beneath the other ventral cells which will become hypoderm or other ectodermal structures (Fig. 3d). The division of neuroblasts involves unequal cytoplasmic division, the smaller products becoming ganglion cells. While this

amnion; cf—cephalic furrow; cpa—cephalopharyngeal apparatus; dbl—dorsal blastoderm; en—endoderm rudiments of mid-gut; fs—frontal sac; go—gonad; hg—hind-gut; ht—heart or dorsal vessel; mg—mid-gut; mp—malpighian tubule; ms—mesoderm; mu—muscle; ns—nervous system; pc—pole cells or germ cells; pr—proctodaeum; pv—proventriculus; sa—salivary gland and duct; sbg—suboesophageal ganglion; se—serosa; sp—spiracle; spg—supraoesophageal ganglion or commissure; st—stomodaeum; tr—trachea; vbl—ventral blastoderm; ye—yolk cells. Only sagittal sections are shown.

process of neurogenesis is going on groups of ectodermal cells invaginate segmentally to give the tracheal pits from which the tracheal system will arise. These provide the

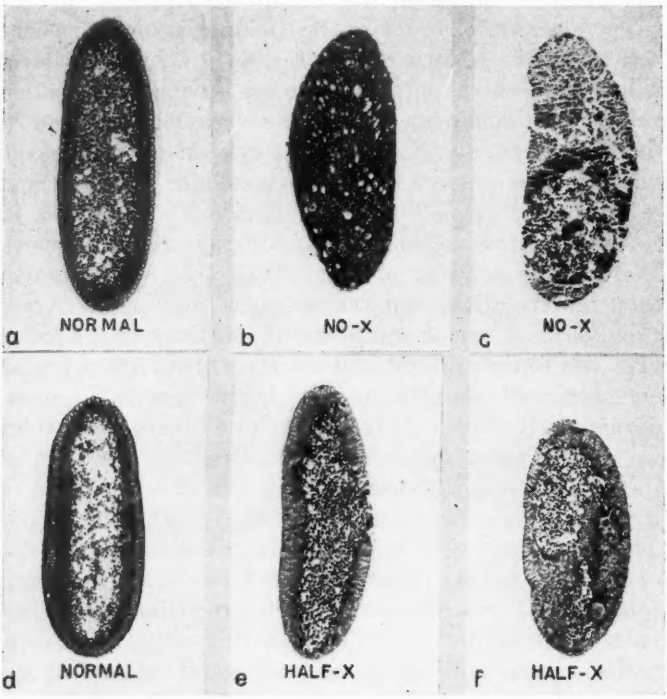

FIG. 4. Early development of normal and deficient zygotes. a.—normal embryo just before the establishment of the blastoderm; b.—nullo-X egg at the end of migration showing nuclei at periphery only in anterior half of egg; c.—nullo-X egg some hours later showing anterior mass of small cells, unincorporated cytoplasm and yolk in posterior part of egg; d.—normal embryo at the time of completion of the blastoderm with cell boundaries fully established; e.—one type of half-X deficient zygote showing incomplete and abnormal blastoderm; f.—the other type of half-X deficiency which becomes abnormal at the time of gastrulation. Photographs of longitudinal sections of iron-hematoxylin preparations. Magnification 112 ×.

first external evidences of body segmentation, except for the boundary between head and trunk which is present from gastrulation on. Anteriorly lateral groups of ecto-

derm cells slowly invaginate to form the salivary glands and become pulled in through the stomodaeum.

The mesoderm spreads out between the ectoderm and the developing gut and later separates into somatic and visceral portions. The two mid-gut rudiments approach each other and eventually enclose the yolk which is surrounded by the yolk cells (Fig. 3c and d). The embryo then undergoes a shortening during which the body segmentation becomes clearly apparent and the posterior end of the embryo retreats from behind the head to its more natural position (Fig. 3e and f). The lateral tissues grow out to complete the dorsal portions of the embryonic trunk.

By this time, 11 to 12 hours after fertilization, the principal processes of morphogenesis and organogenesis are completed and subsequent development consists largely of differentiation. There are no further mitoses in any purely larval tissue after this time. All subsequent growth and differentiation of larval tissues proceeds without cell division. In this period the pharyngeal apparatus, the body and visceral musculature, the dorsal vessel and fat bodies are formed, and the chitinization of skin and tracheae takes place (Fig. 3g). During this period the imaginal discs make their appearance, those of the head being distinguishable first. The fully formed larva (Fig. 3h) hatches from the egg between 20 and 24 hours after fertilization depending on the temperature.

Effects of Large Deficiencies

The removal of an entire chromosome has long been known to be lethal, but this was clearly established for the first and fourth chromosomes of *Drosophila* by Li (1927) in his studies on the effects of chromosome rearrangements. Li did not make any developmental analysis beyond determining that mortality was in the egg stage. If zygotes lacking an entire X-chromosome are studied embryologically (these constitute one fourth of the eggs of attached-X females in *Drosophila*) developmental de-

rangements are first evident in the cleavage stages of the egg (Poulson, 1940a). These lead to an abnormal distribution of the nuclei, which remain principally in the anterior half of the egg (Fig. 4b). Although there is cleavage of cytoplasm to form cells, no blastoderm is produced. The result, after several hours, is an anterior cell mass showing no morphogenetic advances and no differentiation. There is usually a clear stratification of the egg into cellular parts, unincorporated cytoplasm, and yolk (Fig. 4c). Yolk nuclei are frequent, but pole cells are rarely produced. The cells of the anterior mass continue division for some hours, becoming progressively smaller and evidently engaging in little or no synthetic activity. Concomitant physiological changes in nullo-X eggs are discussed below and illustrated in Fig. 8. Such drastic upsets as these, in which the whole pattern for further development is deranged, might well be expected when approximately one fifth of the egg's normal complement of genes is removed from the field of action. What will happen in cases of other deficiencies can scarcely be predicted except that, if these are large, the effects will be early and extensive. This is doubtless true for the large autosomes 2 and 3.

Among eggs produced when the X-chromosome is broken in two by a translocation near its center, T (1:4) A1, also known as the CRB translocation, are some deficient for the region between lozenge and the spindle attachment (Fig. 1) and some deficient for the remainder of the chromosome between lozenge and the left end. In each of these cases the morphological upset is drastic and early, but the two portions of this chromosome have clearly different effects. In one (the X_R deficiency ?) these are somewhat similar to the nullo-X (Fig. 4c) in that the distribution of nuclei is disturbed. This leads to the production of an incomplete blastoderm, the cells of which meet a fate much like that of those in the nullo-X eggs. In the other type of egg (X_L deficient ?) a blastoderm is formed, but separation of the germ layers fails

(Fig. 4f). Morphogenetic movements are abortive and the embryo ends up as a sac of undifferentiated cells. In both instances cell division continues for some hours, although cytological abnormalities are common.

These large deficiencies clearly show that the X-chromosome has a great deal to do with very early embryonic processes, but it is necessary to turn to smaller deficiencies to separate the effects of the individual regions and individual genes.

The Notch and White Deficiencies

Equally significant and no less spectacular effects have been found in instances of very much smaller deficiencies such as can be demonstrated only with the aid of the giant chromosomes of the salivary glands. Of the many deficiencies obtained in experiments by Dr. M. Demerec, of the Carnegie Institution of Washington, the most extensive series are those involving the loci of white and facet near the left end of the X-chromosome (Figs. 1 and 7). Stocks of these provided by Dr. Demerec, whose kindness is gratefully acknowledged, have made it possible to study in detail a region of the X-chromosome which controls certain very fundamental morphogenetic processes (Poulson, 1940a,b; in press). These deficiencies range in size from one removing 45 bands to those in which no visible loss is apparent. Three principal categories of deficiencies are shown in Fig. 7, those which lack both the white and facet loci, and those which lack white only or facet only.

Embryological studies clearly show that the disturbances in each of the three white deficiencies are essentially the same type. Irrespective of the size of the deficiency, organs of ectodermal origin appear to be more or less normal, but those of endodermal and mesodermal nature show clear abnormalities. These become evident between the 12th and 16th hours of development. Instead of transforming into a long convoluted tube the mid-gut remains a large undifferentiated sac without any muscu-

lature, in striking contrast to the normal condition, as in Fig. 5. This failure of differentiation of the gut is correlated with a failure of the body musculature, which begins to be laid down and then degenerates. (The same is true for aorta, fat bodies, etc.) Such embryos, while still alive at the hatching time of normal larvae, never emerge from the egg, and the yolk remains largely unutilized in the mid-gut. This is evidence that a gene or genes in the region of 3C1–3C2.3, which is that common

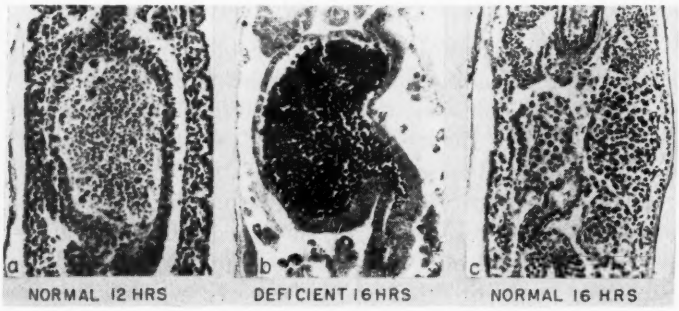

FIG. 5. The abnormal gut and mesoderm of a white-deficient embryo compared with normals. a.—frontal section of a normal embryo at 12 hours showing the mid-gut as a large sac and the beginning of the differentiation of the body musculature to the right; b.—frontal section of a deficient embryo at 16 hours showing only slight morphological advance in the gut, the absence of gut musculature and degeneration of body muscles; c.—frontal section of a normal embryo at 16 hours showing convolutions of mid-gut and presence of gut musculature. Photographs a. and c. from Bodian preparations, b. from iron-hematoxylin preparation. Magnification 272 ×.

to all three deficiencies, is concerned in the development and differentiation of mesoderm and endoderm.

The facet, or Notch, deficiencies, Fig. 7, all lead to an earlier and more drastic series of disturbances in which each of the germ layers is involved. The most conspicuous of these abnormalities is that far too many neuroblast cells are formed from the ventral and cephalic ectoderm so that there is little or nothing left which can give rise to skin and other ectodermal derivatives. The embryo develops a nervous system which has a total vol-

ume of cells at least three times that of normal, Fig. 6. This hypertrophied nervous system shows considerable differentiation, but the patterns of the ganglia and fiber tracts are markedly deranged (Poulson, 1943). There is no condensation such as typifies the later embryonic

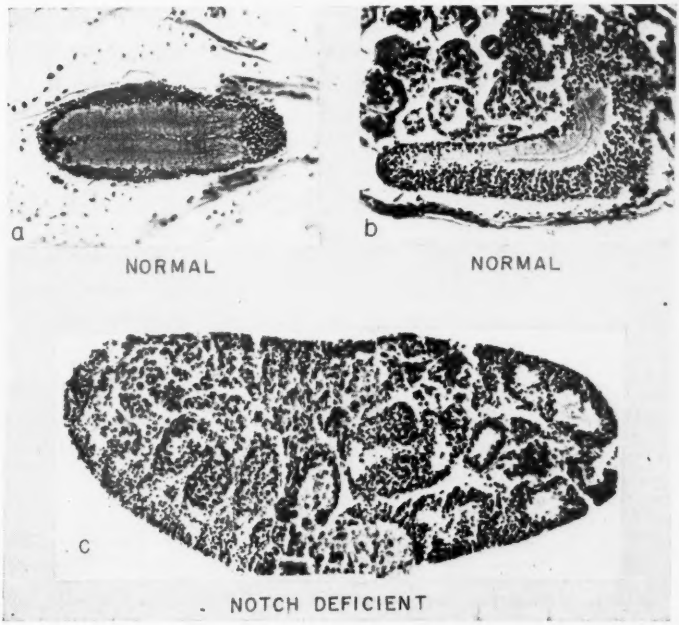

FIG. 6. Notch-deficient embryo and its nervous system compared with the nervous system of normal embryos. a.—frontal section of the highly condensed ventral nervous system of a normal embryo just before hatching; b.—sagittal section of ventral nervous system in a normal embryo of the same age as a.; c.—sagittal section of a Notch-deficient embryo showing the hypertrophied nervous system, absence of ventral hypoderm, incomplete midgut and abnormal mesoderm. Photographs from Bodian preparations. Magnification 310 ×.

period in normal embryos. The nervous system remains naked and uncovered by any outer layer. Only the more lateral and dorsal parts of the hypoderm develop. Tracheal trunks are usually present dorsolaterally and are characteristically chitinized.

The rudiments of the mid-gut fail to unite to form a proper alimentary tract, Fig. 6, and the yolk remains unenclosed by any gut. The yolk mass may occupy any of a number of positions. In Fig. 6c it is seen to lie mid-ventrally, the nervous system being bent around it. The fore-gut is rudimentary and associated structures, such as salivary glands and frontal sac, are lacking. Curiously enough the proventriculus, at the point of union of the fore- and mid-guts, is well developed. The hind-gut is more nearly normal and considerably convoluted. Malpighian tubules are usually present.

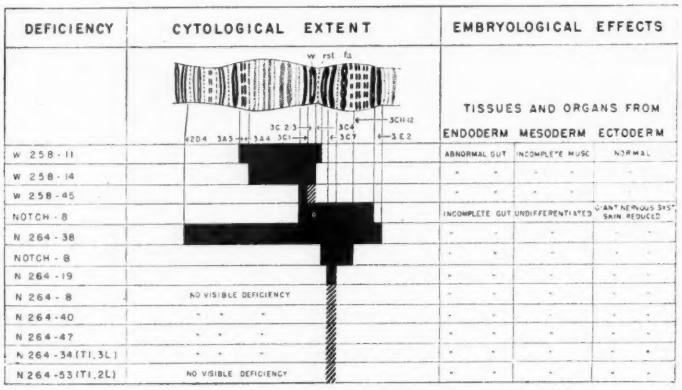

FIG. 7. Summary of the cytological extents and morphological effects of the deficiencies referred to in the text. Data on cytological extent from work of Demerec, Slizynska, Sutton, and the author summarized in Bridges and Brehme (1944). Black indicates visible deficiency; cross-lines no visible detectable deficiency, but definite genetical deficiency.

The mesoderm remains more or less undeveloped, and muscles, heart, fat-body, etc., are not produced. Partially differentiated spindle-shaped cells lying irregularly beneath the dorsal and lateral hypoderm are the nearest approach to muscles. Connective tissue is present.

All in all, a kind of hopeless monster is produced which can not develop beyond the embryonic stage, although its constituent cells and parts remain alive for some hours after normal hatching time. Since the results are the

same both in the larger deficiencies and those which show nothing visibly missing, it is concluded that in all probability a single gene, which may be the normal allele of facet, is the controlling agent of the processes here deranged. The simplest hypothesis to account for the manifold effects of this gene is to assume that it is normally involved at the time of germ-layer separation and has its effects on the cells of the ventral blastoderm, or possibly only on those along the mid-line, the future mesoderm and endoderm cells. In the latter case, these, when turned under, fail to develop normally and so may influence the overlying ectoderm cells. If this is true it may mean that mesoderm normally plays a role in the induction of the insect nervous system, perhaps comparable in importance to that which it has in vertebrates. Little is known of the factors concerned in the development and differentiation of the arthropodan nervous system and the evidence from the Notch embryos may prove a step in this direction.

Embryos deficient for both white and facet (Notch–8 and N264–38) are monsters like those described above. The embryos of Notch–8 are indistinguishable from any of the other facet deficient embryos, while in the case of the largest deficiency (N264–38) the disturbance is the same, but there is even less differentiation in the various gut regions and tracheae are rarely found. Thus the gene, or genes, at the facet locus controls processes which come into operation at an earlier time than any of those controlled by genes in regions removed by the white deficiencies. Further, none of the genes or combinations of genes within the larger deficiencies appreciably modify these processes.

Two of the Notches which show no visible deficiency are associated with translocations (N264–34 and N264–53) in which the point of breakage is close to, or at, the facet locus. The available evidence in these cases suggests that deficiency for the entire tip of the X-chromosome including facet produces these same effects. Very small deficiencies near the tip of the X-chromosome investigated

by Kaliss (1939) and by the writer (Poulson, 1940a) produce their effects very late in the embryo. Other deficiencies in this region reported by Demerec and Hoover (1936), Muller (1935), Sturtevant and Beadle (1936) and Sutton (1940) are the exceptional instances in which both the homozygote and hemizygote are viable and fertile. Thus it would appear that of all loci at this end of the chromosome facet influences embryogenesis earliest, and most extensively, then white and later the other loci. Other genes between bands 3E2 and 8B1 when removed with facet, 3C7, do produce an even greater dislocation of development as indicated in the X_L deficiency. The significance of these loci remains to be investigated with the aid of Notch deficiencies extending further to the right than any now available and with small deficiencies between facet and the locus of lozenge.

It is thus clearly demonstrated that different genes in the X-chromosome have quite different and often clearly separable embryogenetic effects. The individual and additive properties of most of these genes remain to be investigated step by step and related to the structure and chemistry of the chromosome.

THE PHYSIOLOGICAL EFFECTS OF DEFICIENCIES

A first step in relating the developmental effects of chromosomes to cellular physiology, in an organism whose cellular physiology is little known, is to obtain some knowledge of the overall metabolic activities in the developing zygote. Students of the physiology and biochemistry of development have been forced to make studies on large numbers of small eggs in order to have adequate material for the methods available. Provided the materials are uniform no special difficulty presents itself. When, however, there is more than one genetic type among the zygotes it becomes imperative to investigate single eggs. The perfection of the Cartesian diver ultra-microrespirometer (Boell, Needham and Rogers, 1939; Boell and Needham, 1939) provided a means of

determining the respiratory metabolism of single eggs of *D. melanogaster*.

A study of the oxygen uptake of normal fertilized eggs

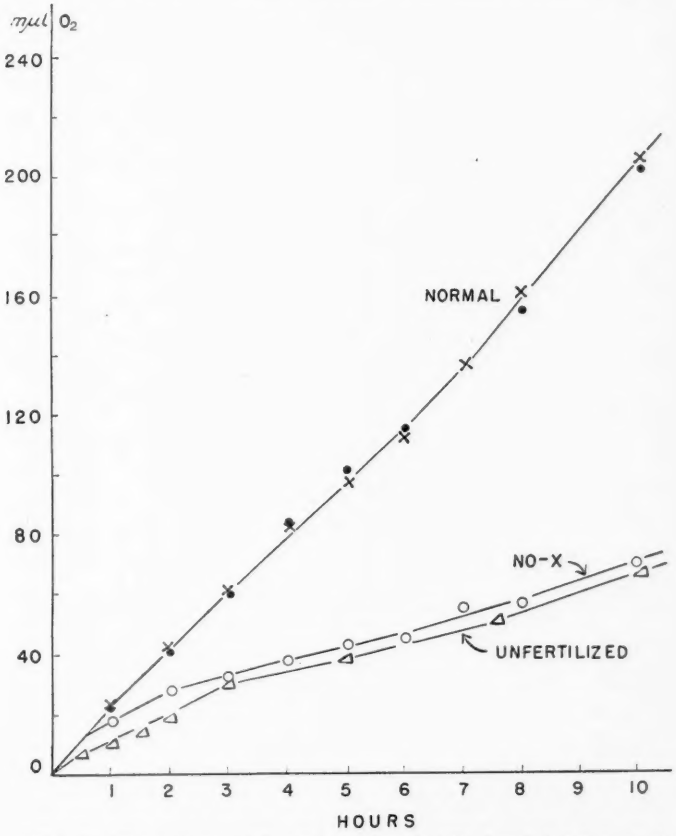

FIG. 8. The oxygen uptake of normal, unfertilized and deficient single eggs of *D. melanogaster* determined with the Cartesian diver ultramicro-respirometer. The time is in hours after the beginning of the experiment which was seldom less than an hour after eggs had been laid. The oxygen uptake is given in millimicroliters at 25° C.

of *Drosophila* (Boell and Poulson, 1939; in press) shows that the consumption is almost uniform with time, having a slight tendency to increase with age during the first

twelve hours of development. The rate of oxygen uptake averages 0.026 cu. mm. per egg per hour in the Oregon R wild strain, no sex difference being distinguishable. The rates in normal non-deficient eggs of other strains are of the same magnitude, but significantly different from the Oregon R wild strain. The respiratory rate of unfertilized eggs is lower from the beginning than that of normal eggs, the final value being 0.005 cu. mm. per egg per hour.

Among eggs from attached-X stocks (of which one fourth are nullo-X), the curves for individual oxygen uptake fall into two groups (Fig. 8), that in which the oxygen uptake is as described for fertilized eggs, and one in which it is much lower. Upon microscopic examination eggs with a normal oxygen uptake have always proved to be developing normally, those with a lower rate to be either unfertilized eggs or nullo-X zygotes. In eggs deficient for the X-chromosome respiration is indistinguishable at the outset from that of normal eggs. Several hours after fertilization the oxygen consumption has declined to a value one fifth that of normal eggs. This is maintained for at least 14 hours at a steady rate which is similar to the equilibrium value of unfertilized eggs. The period in which the rate of oxygen consumption is dropping coincides in time with the appearance of the developmental abnormalities which characterize the nullo-X zygotes. The experimental data indicate that while developmental upset is accompanied by failure in respiration, the mechanism for oxygen uptake in deficient eggs is fully functional for a time immediately following fertilization. Furthermore, the cytological studies on nullo-X referred to above (Poulson, 1940a) show that cell division continues for some hours after the oxygen uptake has dropped to the final value. This raises a number of questions about the role of oxygen uptake in cell division which can not be discussed fully here (see Needham, 1942). What is clear is that cell division, though not increase in cell size or restoration of nuclear size (which means syntheses), can go on when the oxygen consump-

tion is only one fifth that of normal eggs and of the same magnitude as in unfertilized eggs showing no signs of developmental activity. This suggests than an entire enzyme system has become inactive or has ceased to exist in the nullo-X zygotes. A comparison with the situation in other forms such as the echinoderms where Runnstrom (1930) showed that the respiration of unfertilized eggs is not sensitive to cyanide and is non-ferrous while that of fertilized eggs, proceeding at a much higher rate, is so, inevitably arises. There is also a parallel in the difference between normally developing and diapause eggs of the grasshopper elucidated by Bodine and Boell (1934). Until experiments with respiratory inhibitors have been carried out on the several types of *Drosophila* eggs we can say nothing more than that it is very probable that the difference between fertilized and unfertilized eggs which has been found is due to the inactivity or absence of the cytochrome, or Keilin-Warburg system, in the latter. The difference between normal and nullo-X zygotes is probably the same except that the system appears to be present and functional immediately following fertilization and goes out of action simultaneously with the appearance of the embryological disturbances in the deficient eggs.

Metabolic studies on the smaller deficiencies of the white-facet series have progressed only far enough to say that there are no gross respiratory differences such as just described. It seems unlikely that in these embryos in which morphogenesis and differentiation are proceeding, albeit abnormally, any large or overall effect on the respiratory metabolism would be discernible. The biochemical basis of these abnormalities will have to be approached by other methods.

Interrelations of Chromosomes and Cellular Physiology in Development

From the embryological and physiological observations which have been presented it is clear that the chromo-

somes are as much concerned in the processes of development and in cellular physiology as they are in hereditary transmission. This is precisely the picture derived from the *Neurospora* studies, and both emphasize the intimate relations between the chromosomes and the enzyme systems of the cell. In *Neurospora* the correspondence between individual genes and enzymes controlling unit processes in synthesis is very close. In *Drosophila* somewhat similar, but less direct, relations between genes and enzymes are indicated, especially by the investigations on the physiology of eye pigmentation (*cf.* review by Ephrussi, 1942). In all instances the steps between the processes of cellular physiology and the visible ones of development and differentiation require elucidation.

How do genetically identical cells become differently differentiated? Relative positions and gradients (Child, 1941), by themselves, account for some differences and may result in changes in the physiology of a cell, but are not an adequate explanation for so many cases and types of highly specific differentiation. The relations between the chromosomes and units, or elements, in the cytoplasm are most certainly concerned in these cellular specializations. Wright (1941, 1945) has considered theoretically the ways in which genes and cytoplasmic units may be interrelated, and Rhoades (1943) and Sonneborn (1943) have provided specific instances in *Zea* and *Paramecium*. There are in *Drosophila* cases not unlike that which Sonneborn has presented. One is the sex-linked mutant fused (Fig. 1) studied by Lynch (1919). Homozygous fused females produce somewhat fewer eggs than heterozygotes and normals. When fertilized by the sperm of fused males (which are of normal fertility) the eggs of fused females, although they show some signs of embryogenesis, do not produce viable larvae. If fertilized by sperm from normal (not-fused) males they give rise to heterozygous females and normal (not-fused) males, but the fused males die as embryos. The nature of these developmental disturbances remains to be investigated

with embryological methods. Another sex-linked mutant, rudimentary, differs from fused only in degree in this respect. Clancy and Beadle (1937) showed that the ovarian eggs of fused females and those of the mutants singed and female-sterile[2] are uninfluenced by the host when transplanted into normal females in larval stages. Thus no diffusible substance is involved within the stages investigated. The simplest hypothesis is that of non-diffusible plasma genes, or primers, controlled or synthesized by the chromogenes, or nuclear genes, and transmitted through the cytoplasm of the egg. These are exhausted before egg production in singed and female-sterile, but persist in the fused female. Introduction of the normal nuclear gene through a normal sperm into the eggs of fused females starts the synthesis again. Thus the case of fused, as well as other instances of female sterility in which eggs are produced, deserves a thorough biochemical-embryological investigation. Whether any of these mutants may be due to cytological deficiency remains to be established, but it seems unlikely in certain of the cases, such as singed, in view of the numbers of alleles some of which are normal or approach normal in fertility.

Direct evidence of a control of the cytoplasm by the chromosomes is found in the nullo-X eggs (Fig. 4), where there is a breakdown of the cortical layer at the posterior end of the egg at and following the time of migration of the nuclei (Poulson, 1940a). Thus the functional integrity of the cytoplasm depends on the presence of the nucleus in these early stages.

Since there is clear evidence in insect eggs of cytoplasmic segregation during development—widely known in the cases of the pole cells and the neuroblasts—Rhoades' and Sonneborn's suggestions concerning the role of cytoplasmic elements in differentiation apply particularly well to *Drosophila*. It is not difficult to visualize

[2] Eggs of singed females are abnormal in shape and size and do not develop; those of female-sterile homozygotes never become mature. The latter case may be different in nature from the others cited.

how the development of a tissue such as the mesoderm, in the white-deficient zygotes (Fig. 5), might proceed normally up to the point at which the primer normally comes into play and then cease differentiating or even de-differentiate. The hypertrophied nervous system of the facet-deficient embryos might arise through a disturbance of cytoplasmic segregation at the time of determination of neuroblasts in the ventral ectoderm, just as well as by the overzealous organizer activity suggested previously. A precise application of this point of view in the analysis of the white and facet deficiencies should prove most illuminating, but will not be attempted here because of limitations of time and space.

Advances in our knowledge of the chemical nature of the chromosomes (Mirsky, 1943; Mirsky and Pollister, 1942, 1943; Gulick, 1944) and of certain cytoplasmic components (Claude, 1941, 1943) demonstrate that the former consist primarily of desoxyribosenucleohistones while the latter are in large part ribosenucleoproteins. The absence of the desoxyribosenucleic acids (Mirsky and Pollister, 1943) from the cytoplasm and the presence of the ribosenucleic acid in both nucleus and cytoplasm suggests that the synthesis of the latter takes place in the nucleus under the influence of the chromosome (Caspersson and Schultz, 1940). The discovery that a desoxyribosenucleic acid is the active substance in the transformations of pneumococcal types by Avery, MacLeod and McCarty (1944) indicates that such substances possess a high degree of specificity and structural complexity. The nucleotide or nucleoprotein nature of many enzymes or coenzymes and the intimate association of some of them with cytoplasmic structures fits very well into the general picture of a system of syntheses. How other types of enzymes such as those of the cytochrome system may be related to this is an important problem.

Ordinarily we do not determine the presence or absence of an enzyme directly, but only by whether or not a reaction or a step in a reaction which it catalyzes is completed.

In both *Neurospora* and *Drosophila* the enzymes in gene controlled reactions are far from being identified. The biochemical mutants of *Neurospora* are maintained by supplying the missing products of synthesis. Whether the deficient zygotes in *Drosophila* can be made to develop if supplied with missing organic molecules or enzymes remains for the future. Even if this be accomplished there will remain unsolved the basic problem of how to pass from a knowledge of the nature of the secondary products of the chromosomes to the intimate structure of the chromosomes themselves, of integrating the isolated systems into the whole dynamic structure. It is a firm conviction of the writer that the patterns of development and differentiation of the organism are implicit in the functional architecture of the cell which in turn has its foundations in the ultimate structure of self-reproducing nucleoprotein systems, chief of which are the chromosomes. Only by filling in the patterns step by step and from level to level, while keeping clearly in mind that there are no boundaries in biological or other science—that genetics, embryology and biochemistry are all approaches to the same thing—can this difficult gap be bridged.

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REVIEWS AND COMMENTS

EDITED BY PROFESSOR CARL L. HUBBS

IN these reviews and notices of current biological publications emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution. REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as indicated, all items are prepared by Dr. Carl L. Hubbs, Scripps Institution of Oceanography, University of California, La Jolla, California. All opinions are those of the reviewer.

A Story Outline of Evolution. By CHARLES W. GRIMES. Boston: Bruce Humphries, Inc., 1945: i–viii, 1–244. $2.00.

THE announcement of this book rightly states that "for years the principles of evolution have stood in sore need of intelligible popular explanation." There can be no doubt that the "Story Outline" is popular in the sense of being nontechnical and unprofessional, and it is reasonably intelligent. Chief doubts arise as to whether it constitutes an adequate explanation.

Despite its deficiencies this volume will undoubtedly free many a mind from the shackles of fundamentalism, and in so doing will fulfil an obvious intent. The presentation was clearly meant to convince the religiously minded of the truth of evolution. Thus the first half of the book is devoted to a discussion of the evolution of human culture and of the machines and gadgets that go with it. Much of this is so self-evident and so generally understood as hardly to need exposition, except in that it puts the reader into the habit of thinking in developmental terms. The argument for organic evolution in the second half starts with a rebuttal of the literal interpretation of *Genesis* and even quotes William Jennings Bryan to the effect that truth must prevail, even though it may lead to the idea that man is a development of beasts. Factual statements, whether true or untrue, are seldom dimmed with any qualification, and assumptions, no matter how novel or how personal, are presented with the same force and with as little equivocation as are the supposed facts. Such tricks of writing may well enhance

the effect of the book on those untrained in biology, for most people think in terms of examples, reason from analogy and seldom qualify their evidence.

The author writes with deep sincerity and great conviction, in fact with almost as much fervor as the religious bigot displays. He seems merely to have replaced an old and narrow faith with a new faith that stems from something other than a thorough understanding of nature. The lack of biological background is almost continuously evident in the discussion of organic evolution, for there is scarcely a page that does not reek with an error or a contradiction that a good biologist could have corrected. Misstatements are not infrequent in the other sections of the book as well.

Disregarding obvious contradictions, bits of long obsolete information and absurd assumptions I select as condemning evidence the following assertions: There are millions of electrons within each atom and millions of atoms within each molecule. The great glaciers moved from Alaska to the Ohio River. Bacteria are animals. The course of plant evolution is "from the algae to mosses, from mosses to ferns, from ferns to shrubs and plants and from plants and shrubs to trees." All plants reproduce through different members of the body, but in animals this process is the result of the function of different and separate bodies. There were 1,700 families of trilobites. Trilobites were the ancestors of crabs, and sea scorpions became land scorpions. The nerve conductors of the lower forms gradually wove themselves into a notochord, which in turn changed into a jointed backbone. The scales of fishes changed into the hair of mammals. Snakes paralyze their victims by a piercing glow of the eye. Milk is distilled from food. No differences in form, size or composition are apparent between the germ cells of man and of any mammal. The Chinese have an alphabet. And so on *ad nauseam*.

Preordination carried back to the Creator is the keynote. The entire course of evolution, down to the fine

details, is pictured as having followed the Universal or Divine Plan of Creation. God crowded into a single cell the attributes of all plant and animal life. Plants came into being first, to add beauty and fragrance to the world and to provide also for the more corporeal needs of animals, that were to appear later. The plants "evoluted" to these ends by reason of their own intelligence, "but it is not given to our minds to understand the cunning of plant intelligence." In turn all animals arose so that, through the ordained channels, they might contribute to the origin and perfection of man.

As though such thoughts transcend the methods of evolution natural selection is scarcely considered. At times the Lamarckian viewpoint is expressed as though it were the accepted tenet of biologists. New varieties of plants have been created by cultivating and fertilizing the soil and by budding, grafting and pruning! Forelimbs developed into wings by their constant flapping! Man lost the delicate sense of smell through disuse! The work of an artist may be the result of electronic forces generated by a vision in the brain cells of a remote ancestor! (Possibly that is true of some modern art.)

A Preliminary Analysis of the Herpetofauna of Sonora. By CHARLES M. BOGERT AND JAMES A. OLIVER. Bull. Am. Mus. Nat. Hist., 83, 1945: 297–426, pls. 30–37, figs. 1–13, maps 1–2. $1.00.

To an increasing degree systematists are bringing their faunal and revisionary studies to bear on the more general problems of distribution and speciation. The paper under review is a good example of this trend. It is based on an analysis of the 125 kinds of amphibians and reptiles known from Sonora. The "herpetofauna" of this Mexican state is pictured as a mixture of "Old Northern" (pre-Holarctic) and "South American" types. From an unpublished study I can say that this is true also of the fresh-water fishes of northwestern and also of northeastern Mexico, but I would look on the "South American" elements largely as Middle American developments with

a long period of endemic evolution. Routes of faunal immigration are indicated as from the south, along the coastal plain, from the Colorado Desert to the northwest, from the plateau to the east through mountain passes, and along the mountains from the south and from the north. The fishes obviously came mostly from the south by the coastal plain, to a lesser extent from the plateau through stream capture, and from the mountains to the north.

The Colorado Desert is indicated as an effective barrier and it is suggested that the greatest effect has been on older stocks with reduced adaptability. Though this great desert gives evidence of having been of long standing, the disjunct distribution of moisture-living types in Sonora is taken as evidence of a geologically recent moist period. In sharp contrast to Epling's view that the temperatures in the region were not markedly reduced in the Pleistocene, Bogert and Oliver infer from present distributional patterns that Pleistocene cold forced animals southward into the peninsulas of Baja California and Florida and the "paleopeninsula" of the Mexican plateau, there to isolate populations and thus to permit speciation. I suspect that the actual temperatures far south of the ice sheet lay between the extremes postulated by Epling and by Bogert and Oliver. It seems unlikely that entire populations were forced into Baja California by the cold, but many populations no doubt did enter the peninsula and did undergo differentiation there. Such speciation, however, may not have been solely of the peninsular category, for there is evidence indicating that much of Baja California was a large island in Pleistocene or perhaps Pliocene time.

From a herpetological basis it is concluded that exclusively areal, non-disjunct biotic provinces or regions are of limited value. "More can be gained from plotting life zones and dispersal routes than by attempts to bound biotic provinces." This criticism of a modern trend has much justice, for the biotic provinces as mapped are too

generalized to be of much aid in the explanatory phases of either biogeography or speciation.

There are other data of general speciational as contrasted with herpetological interest, but these data are largely buried in the systematic account.

ARTICLES AND SHORTER BOOKS

Root Disease Fungi. A Treatise on the Epidemiology of Soil-borne Disease in Crop Plants, and a First Exposition of the Principles of Root Disease Control. By S. D. GARRETT. Annales Cryptogamici et Phytopathologici, I. Waltham, Mass.: The Chronica Botanica Co. (New York: G. E. Stechert and Co., agents), 1944: i–xvi, 1–177, figs. 1–9. $4.50.—"The root-infecting fungi, as an ecological group, afford a wealth of material to the student of evolution; to the plant pathologist, they offer a series of intricate problems in applied biology." It is obvious that the phytopathological aspects have been of most concern to the author, and it is to be hoped that he may follow through with a discussion of the evolution of these organisms. He does devote one chapter to parasitic specialization in the root-infecting fungi. The conclusion is reached that parasitism in the soil invaders arose from the saprophytic habit of the soil inhabitants. He adds that "if symbiosis be regarded as the evolutionary end-point of the parasitic habit, then the mycorrhyzal species must be regarded as the most highly specialized of all the root-infecting fungi. The parasitic habit of these plants and other phases of their ecology and physiology are then treated, as a background for the chapters on root disease control. This is an important, well-written and pioneering volume. Like other Chronica Botanica books it is neatly made.

Flora of Illinois. By GEORGE NEVILLE JONES. Notre Dame, Indiana: The University Press (American Midland Naturalist, Monograph Series, Monograph No. 2), 1945: i–vii, 1–318, 1 fig., 2 maps. $4.00.—To collect, prepare and identify 15,000 specimens, to extend this study to the previous collections deposited in such herbaria as those of the University of Illinois, the Missouri Botanical Garden and the Field Museum as well as several private herbaria, to organize and compile keys to these collections from a bibliography of over 400 listed items and to write and

prepare the manuscript for publication is a colossal job on the flora of any state. That all of this can be accomplished in five years is attested by the published volume, Flora of Illinois, by George Neville Jones. With a very brief introduction of a geographic and ecological nature the main work follows in the form of synoptical keys including what there is of range, habitat, local restriction and seasonal periodicity. Brevity has been the key note. The work treats 152 families, 716 genera and 2124 species of vascular plants. The arrangement is that of Engler and Diels. Botanists will especially welcome the taxonomically classified bibliography and the originality in the glossary.—HERBERT L. MASON, Department of Botany, University of California, Berkeley, California.

Fragmenta Papuana. Observations of a Naturalist in Netherlands New Guinea. By H. J. LAM. Translated from the Dutch by Lily M. Perry. Sargentia (Arnold Arboretum of Harvard University, Jamaica Plain, Mass.), 5, 1945: i–iv, 1–196, figs. 1–32, maps A–B. $3.00.—Geographical, hydrographic, vegetational, floristic and some zoological and ethnological data are crammed into these interesting accounts of explorations in New Guinea.

Scientific Results of Cruise VII of the Carnegie during 1928–1929 under Command of Captain J. P. Ault. Oceanography—II. I. Marine Bottom Samples Collected in the Pacific Ocean by the Carnegie on Its Seventh Cruise. By ROGER R. REVELLE. II. Radium Content of Ocean-Bottom Sediments. By CHARLES S. PIGGOTT. Carnegie Inst. Wash. Publ. 556, 1944: i–x, 1–196, 14 pls., 47 figs., 12 charts. $2.00 (paper), $2.50 (cloth).

Chemistry—I. Chemical Results of the Last Cruise of the Carnegie. By HERBERT W. GRAHAM AND ERIK G. MOBERG. *Ibid.*, 562, 1944: i–vii, 1–58, 24 figs. and charts. $1.00 (paper), $1.25 (cloth).

Biology—V. The Genus Ceratium in the Pacific and North Atlantic Oceans. By HERBERT W. GRAHAM AND NATALIA BRONIKOVSKY. *Ibid.*, 565, 1944: i–vii, 1–209, 27 figs., 55 charts. $2.00 (paper), $2.50 (cloth).

FIFTEEN years after the last cruise of the *Carnegie* came to a

disastrous end there are now being published some of the contributions to oceanography that resulted from this finely planned and well-executed expedition. In studying the ocean bottom samples Roger R. Revelle made use of modern methods of sedimentational and soil analysis. The objects were "(1) the determination of the various constituents of the samples with the view of ascertaining their physical and chemical nature, origin, and history before and after deposition; and (2) the classification of the samples from the point of view of the causes of the variation in bottom deposits with depth and location." The attainment of these objects will go far toward clarifying many problems in the history of the earth and in the distribution and evolution of sea life. The data, however, are very complex, and grave difficulties are encountered in arriving at objective conclusions. Factors responsible for the deposition of calcareous matter are particularly complex. The author leans toward the view that much of the firm clay on the ocean floor is of continental origin. In the short supplement Piggott reports 28 analyses of the high radium content of deep-sea deposits and reviews the few previous analyses that have been made.

In the chemical report chief attention is given to the distribution of phosphate, silica, hydrogen ions and dissolved oxygen. The relative high phosphate content of the deep Pacific and the Antarctic waters is emphasized and explained in dynamic terms. In silica content the Pacific also seems to exceed the Atlantic. In pH content the Pacific and Antarctic waters further agree and contrast with Arctic and North Atlantic waters. The very low dissolved oxygen content in the deep Pacific is taken as evidence that these waters have lacked contact with the surface layer for a greater length of time than has the deep water of the Atlantic.

In the monograph on the dinoflagellate genus *Ceratium* Graham and Bronikovsky describe regional differences in species numbers, discuss factors affecting horizontal distribution, compare the Atlantic and Pacific *Ceratium* floras, and treat the vertical distribution of these organisms. They then present extensive ecological data for each species, and supplement their account with long tables of data, many figures showing differences between and variations within species, and finally charts showing (1) the horizontal and vertical distributions of many species, (2) surface temperatures, salinities and phosphate content, (3) the clear-cut zones of species abundance, and (4) the *Ceratium* life zones.

Altogether these reports constitute a major contribution to oceanography. Their general value would have been increased by the inclusion of clear-cut interpretive summaries.

A Source-Book of Biological Names and Terms. By EDMUND C. JAEGER. Springfield, Illinois, and Baltimore: Charles C Thomas, 1944: i–xxvi, 1–256, illustr. $3.50.—This lexicon is a great improvement over *A Dictionary of Greek and Latin Combining Forms Used in Zoological Names*, by the same author, for it is much more extensive (now defining "fully 12,000" word elements), deals with botanical as well as zoological names, covers terms more thoroughly, in general gives the correct Classical roots rather than presenting combining forms with little rule or uniformity, and is more replete with interesting examples of names and terms derived from the Classical and other languages. The aim remains the same, to provide a ready means by which those who are not equipped by patience, training or library to use Classical lexicons may understand and appreciate the meaning that might otherwise lie hidden in biological names and terms. It is definitely not a manual for use in coining names, though the introduction briefly explains how words are built and quotes Palmer's interesting essay on the formation of generic names. The Greek alphabet and its equivalents and the rules for transliteration from Greek to Latin are useful features, though throughout the text the transliterating has already been done. In these days of limited or abandoned Classical training this source-book should prove useful to biological students.

Get More Out of Life! By ADRIAN J. GILARDI. Boston: Bruce Humphries, Inc., 1944: 1–192. $2.50.—With a totally non-biological view an engineer presents his thoughts on man's contact with his environment. It may be good ethics, or good medicine for sick minds.

SHORTER ARTICLES AND DISCUSSION

ISOLATING GENE E′ FOR EARLY SEXUAL MATURITY[1]

Introduction

Early sexual maturity is an important inherited character affecting egg production. The mode of inheritance has been studied by several investigators. Hays (1924) suggested that one sex-linked and one autosomal gene were responsible for the dominance of early sexual maturity over late sexual maturity. Waters (1934) showed that a sex-linked gene was concerned. Warren (1934) concluded that both a sex-linked and an autosomal gene must be present to produce early sexual maturity. Hays (1936), by crossing early and late maturing strains, demonstrated a cumulative effect between sex-linked gene E and autosomal gene E′. Birds carrying both genes were very early maturing, those having either genes E or E′ alone were medium early maturing, while those lacking either or both of these genes were late maturing. The phenotypes suggested were: very early, under 180 days; medium early, 180–215 days; and late, 216 days or more.

Objects of Later Study

It seemed to be desirable to develop a strain of birds pure for autosomal gene E′ to discover the effect of this gene alone on age at sexual maturity and later to observe the result of bringing together genes E and E′.

Experimental Results

Two Rhode Island Red males believed to be genetically late maturing were mated to Barred Plymouth Rock females whose age at sexual maturity ranged between 190 and 210 days. If the Barred Plymouth Rock females carried the sex-linked gene E they would transmit it only to their sons and never to their daughters. The barring gene and the silver gene would serve as markers of the sex chromosome derived from the Barred Plymouth Rock hens. Except for crossing over in the males, the gene E would remain linked with barring and silver (Warren, 1934).

[1] Contribution 556 of the Massachusetts Agricultural Experiment Station.

Character of F_1 Daughters

The first male gave 12 females with an age range from 179 to 215 days and a mean age of 196 days, group A. The character of the daughters indicates that their sire was a pure recessive ee e′e′ and that the Barred Rock hens were e E′E′ because all daughters were medium maturing.

The second male gave 12 daughters with an age range between 177 and 312 days and a mean age of 211 days, group B. Their phenotypes with respect to age were 1 early; 9 medium; 2 late. This ratio suggests that the sire carried the sex-linked gene E and that the dams were heterozygous for gene E′.

Character of the F_2 Daughters

To produce the F_2 generation an F_1 male from group B was mated to F_1 daughters from group A all of which showed medium maturity. A total of 16 F_2 daughters was produced from this mating. Four of these daughters exhibited barring and silver, indicating that their single sex chromosome had passed unaltered from their Barred Rock granddam. All four of these were definitely late in sexual maturity, having a mean age of 297 days. This indicates that sex-linked gene E was not present in the original Barred Rock females. Since these females were all phenotypically medium in sexual maturity they must have carried autosomal gene E′.

In the F_2 generation four daughters appeared that carried a crossover sex chromosome with silver and lacking barring. Two of these were medium in maturity and two were late. Only one daughter with barring and lacking silver was tested for age at sexual maturity and she matured at 215 days.

Seven daughters carrying their Rhode Island Red grandsire's sex chromosome were tested. These daughters lacked both barring and silver. Two out of the seven showed medium maturity and five were late maturing. If their grandsire had carried gene E, no less than one half of these females would have shown the effects of gene E and extra early sexual maturity would have appeared in some daughters because of combined effects of genes E and E′.

A second group of F_2 females was produced by mating an F_1 male from the group described in the preceding paragraph to F_1 females descending from a Rhode Island Red male that carried sex-linked gene E mated to Barred Rock females that were hetero-

zygous for autosomal gene E′. The F_1 daughters tested showed 1 very early to 9 medium early to 2 late maturing. Two of these late-maturing females were mated to the F_1 male. Two daughters were tested and one carrying barring and silver as contributed by the sex chromosomes of the grandmother showed medium maturity because of gene E′. The other showed late maturity. From the second mating to a late maturing F_1 female two daughters carried early maturity. One carried the original sex chromosome of the grandmother BS and the other inherited her sex chromosome from her grandsire bs. There were two late maturing daughters each with a bs sex chromosome.

Three medium maturing F_1 females from this same group were mated to the F_1 male. A total of seven daughters was tested for age at sexual maturity. The result was 5 medium to 2 late. Since no very early maturing daughters appeared it is probable that both the sire and dams lacked gene E and were heterozygous for gene E′. This would give a 3 to 1 ratio which was closely approached. Only one medium maturing daughter carried barring and silver. One of the late maturing daughters carried barring and silver and the other was a crossover with silver alone.

Character of the Third Generation Daughters

An F_2 male from group B females was mated to five phenotypically late-maturing Rhode Island Red females with an age range from 257 to 298 days. This F_2 male was of a medium light shade of Rhode Island Red color and carried neither barring nor silver. It is very probable that neither of his sex chromosomes came from his Barred Rock grandmother. He evidently carried only gene E′ for early sexual maturity. From this mating appeared 11 medium early daughters and 11 late daughters exactly as expected. The mean age of the medium early daughter carrying gene E′ was 200 days and the mean of the late daughters was 307 days.

A supposedly late-maturing Rhode Island Red male was mated to two F_2 females that lacked barring and silver. One dam U715 matured at 254 days and gave when mated to the Rhode Island Red male 3 medium (mean 199 days) daughters to 4 late (mean 252 days). This same male mated to U1115 maturing at 203 days gave two medium to two late daughters. The Rhode Island Red male selected must have carried gene E′ because he gave equal numbers of medium and late daughters from a late dam

and because he gave no very early maturing daughters from a dam transmitting gene E′.

Character of the Fourth Generation Daughters

One solid black male lacking barring and silver and hatched in the F_2 generation was mated to five females produced in the third generation. These females were sired by an F_2 male mated to late Rhode Island Red females. Two of these females were phenotypically medium early in maturity (200 and 210 days, respectively). From the first of these hens all eight daughters were phenotypically late, but this appeared to be due to pathological conditions. The second female gave 8 medium early (mean 201 days) and 3 late (mean 249 days). The expectation would be $\frac{3}{4}$ medium $\frac{1}{4}$ late if both parents were heterozygous for gene E′.

This same black male was also mated to three phenotypically late-maturing females (338, 307 and 334 days). Nine daughters were tested of which four were medium (211 days) and five were late (273 days). This ratio suggests that the male carried gene E′ in heterozygous condition.

Character of Fifth Generation Daughters

A male of the fourth generation was mated to four of his full sisters to make a generation essentially homozygous for gene E′. All sisters selected showed medium early sexual maturity. From these matings 14 daughters were tested and all showed medium early maturity with a mean age of 213 days. The mean of 213 days is somewhat greater than previous means for females carrying gene E′ and the increase in age may be due to inbreeding (Hays, 1924).

A male of the fourth generation from a late maturing dam was chosen and mated to a group of his full and half sisters. So few progeny resulted that no daughters were tested for age at sexual maturity.

Characters of the Sixth Generation Daughters

A male was selected from a family of Rhode Island Reds that carried genes E and E′. The family of 19 sisters to this male had an age range from 156 to 203 days with a mean of 178 days.

This Rhode Island Red male was mated to two very early maturing Rhode Island Red females (163 and 166 days) from

which daughters were tested. A total of six daughters was tested and they showed a mean age of 184 days. The sire was evidently Ee E′E′ or Ee E′e′ in genetic composition and the two dams were probably EoE′E′ because they gave 3 very early to 3 medium early daughters as would be expected from such a mating. The mean age of the very early daughters was 175 days and for the medium early the mean was 192 days.

This Rhode Island Red male was also mated to three medium hybrid hens (212, 215 and 203 days). These hens carried only gene E′ for which they were homozygous. The mean age of the five daughters tested was 182 days. There were 2 very early to 3 medium early daughters, as expected. The mean age of the very early daughters was 172 days and of the medium early 190 days. There is thus very excellent agreement in results from mating one male to two kinds of hens.

A hybrid male known to carry gene E′ alone was mated to two very early maturing Rhode Island Red females (168 and 156 days). Four daughters tested from these matings had a mean age of 181 and all were medium early maturing as expected.

This hybrid male was also mated to two medium maturing Rhode Island Red females (206 and 208 days). Five daughters were tested from these matings and their mean age was 198 days and all were medium early.

SUMMARY

By marking the sex chromosome with such dominant genes as barring and silver it is a relatively simple matter to eliminate the sex-linked gene E for early sexual maturity in hybrids between Barred Plymouth Rocks and Rhode Island Reds. When the sex-linked gene is eliminated it is not difficult to develop a strain homozygous for autosomal gene E′ alone. This procedure has been followed and a line pure for gene E′ was later crossed with a line carrying both genes E and E′.

It was observed that the mean age at sexual maturity of groups of daughters carrying gene E′ alone usually ranged from 190 to 200 days under the conditions of the experiment. When genes E and E′ were brought together the mean age was reduced to from 170 to 175 days. When both genes were absent the mean age at sexual maturity was variable with means of groups of daughters ranging from 250 to 300 days.

F. A. HAYS

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SEX DETERMINATION IN TRITURUS VULGARIS LINN (TAENIATUS SCHNEID)

The genetics of sex determination in Urodela are far from understood.

Humphrey (1942, a, b) converted a genetically female individual of *Amblystoma mexicanum* into a male by a testis implant which was later removed. With normal females it gave about 3 females to 1 male, and while about two-thirds of these females gave a normal sex ratio with normal males, one-third gave all female progeny. The 3:1 ratio was also observed among the offspring of two genetically female *A. tigrinum* similarly changed into males. Humphrey concludes that the male is XX, the normal female XY, and the thelytokous female YY. If so, the X and Y chromosomes can be little differentiated, femaleness behaving as if due to a gene which is completely dominant, at least in diploids. Such a simple sex-determining mechanism is clearly less stable than one in which the YY homozygotes are inviable, and we should expect to find different mechanisms in related species, as in the Cyprinodont fishes.

Fankhauser (1940) found that a triploid *Triturus viridescens* male was normal, while females had poorly developed gonads. This was confirmed by Griffiths (1941). Fankhauser concluded that the males are XX or XXX, females XY and the undeveloped triploid females XXY.

But in *Triturus pyrrhogaster* the triploids described by Kaylor (1943) were all females with well-developed gonads, suggesting that the female is here homogametic. The comparable evidence regarding the mechanism of sex determination in *Triturus vulgaris* so far rests on two animals, an adult functional triploid male found by Book (1940) in Sweden and a haploid female described by Fankhauser (1938), and obtained experimentally while working in Switzerland. These are explicable if the male is X, XX or XXX, the females Y, XY or YY, as would be expected if the mechanism were the same as in *Amblystoma mexicanum*.

There are however, two further pieces of circumstantial evidence concerning *Triturus vulgaris*. Pariser (1932) by means of artificial fertilization obtained offspring from the cross *T. vulgaris* ♀ × *T. cristatus* ♂. Of these 121 contained ovaries, 1 contained testes and in 1 the gonads were undifferentiated. A great preponderance of one sex is commonly observed among species hybrids and very few exceptions have been found to "Haldane's rule" (1922) that the sex which is deficient is the heterogametic sex. This therefore strongly suggests that in both *T. vulgaris* and *T. cristatus* the female is normally XX.

Van Swinderen (1928) found neotenic members of *T. vulgaris* in 7 ponds in Western Holland, the females being fertile, but the males sterile till after metamorphosis. In one of these ponds a number of the neotenic females were white with black eyes, resembling white axolotls. The gills were bright red, and the tails so transparent that their vertebrae could be counted. No pigment was visible macroscopically. No intermediates were found.

Van Swinderen suggested that the whiteness was due to endocrine disturbances. However, every sharp color variation in part of a population that has been investigated has been found to be gene controlled. With the exception of black-and-white in Ayrshire cattle (Wentworth, 1916) which has different dominance relations to red-and-white in the two sexes, every such variation in tetrapods found only in one sex is due to sex-linked genes, though the action of color genes in *Lebistes reticulatus* is limited to the male. However, a naturalist who found a rare variation confined to females in a bird population would provisionally ascribe it to a sex-linked recessive gene.

Therefore at first sight van Swinderen's observation supports the theory that the female in *T. vulgaris* is XY. However, normal sex linkage is only possible where there is a differential segment between the X and Y chromosomes, *i.e.*, where the Y carries no allelomorph of the locus concerned. But in this species a haploid female (on this hypothesis without an X chromosome) has been fairly viable and was not white. Therefore the differential segment must be small and it is unlikely that it exists at all.

Another explanation of van Swinderen's population can be made by comparing it with the polymorphism of the beetle *Phytodecta variabilis* (de Zulueta, 1925) and the segregation of several *bobbed* mutant allelomorphs in about 3 per cent. of females

in many wild populations of *Drosophila hydei* (Spencer, 1938). These animals show a sexual dimorphism just because both X and Y chromosomes carry the loci concerned.

These loci extremely rarely, if ever, undergo recombination with the sex-determining parts of the chromosomes. Hence a recessive mutation on the X chromosome can give rise to recessive zygotes of the homogametic sex only. Recessives can only occur in the heterogametic sex as the result of independent mutation in the Y chromosome, or of crossing over between the X and Y, if this occurs. A recessive mutation on the Y chromosome can only give rise to recessives in the normally heterogametic sex if it meets a corresponding allele in the X, or if a YY or Y zygote is viable, and is formed as a result of sex reversal or some other abnormal process.

On this hypothesis the appearance of white females is explained by the female *T. vulgaris* being homogametic, and the mutant gene causing whiteness and neoteny having arisen on the X chromosome near enough to the sex-determining gene or region to prevent recombination between the date of the original mutation and of the inbreeding that caused the segregation.

Thus evidence is presented supporting the suggestion that in *T. vulgaris* as in *T. pyrrhogaster* the female is the homogametic sex. If this is so Fankhauser's haploid female would be X and Book's triploid male most likely XYY, though as the sex-determining mechanism is variable within the genus it may even vary within races of the same species (compare Winge's experimental results with *Lebistes*, 1934). Reciprocal interspecific crosses between two species or races which had different homogametic sexes would be expected to produce different sex ratios and degrees of differentiation. The results might be expected to be complicated, as the chromosome pair differentiated into X and Y might not be homologous in the two organisms.

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SOME OBSERVATIONS ON LEAF SHAPE EXPRESSION IN THE MALVACEAE

DEVELOPMENTAL studies of leaf shape in *Gossypium* (Stephens, 1944; in press (c)) have shown that all leaf shapes in the genus pass through, or are potentially capable of passing through, three successive phases: (1) an entire (unlobed) leaf phase; (2) a phase in which the leaves at successive nodes become progressively laciniated into five lobes; (3) a climax phase in which leaf shape is stabilized. This development is controlled by a single multiple allelomorph system. Monogenic control of leaf-shape patterns of a similar kind is found in other genera in the Malvaceae, *e.g.*, in *Malva* (Kristofferson, 1923), in *Hibiscus cannabinus* L. (Deshpande, 1942), and recently evidence of a similar system has been obtained in *Hibiscus rosa-sinensis* L. which will be discussed here.

An examination of the different horticultural strains of *H. rosa-sinensis* grown in Trinidad shows that in most cases the climax leaves are entire. In certain types, however, when the seedlings are pruned back hard enough to encourage dormant buds to develop towards the base of the stem, it is noticed that the first leaves to unfold are lobed. Later, the normal entire leaf is regained. Most ornamental strains of *rosa-sinensis* are highly sterile, but at least two, "Hawaiian White" and "Apricot," set very occasional capsules by open pollination. Seeds from a single capsule of the latter were sown out in order to observe the juvenile leaf stages. Eight seedlings were raised, of which three

were entire leaved throughout their life history. In the remaining five plants, the first leaves were entire, but subsequent leaves became progressively more laciniated until about the tenth node. Later, secondary lobes were developed which progressively obliterated the main sinuses and at the flowering stage most of the leaves had become secondarily entire. These plants could be

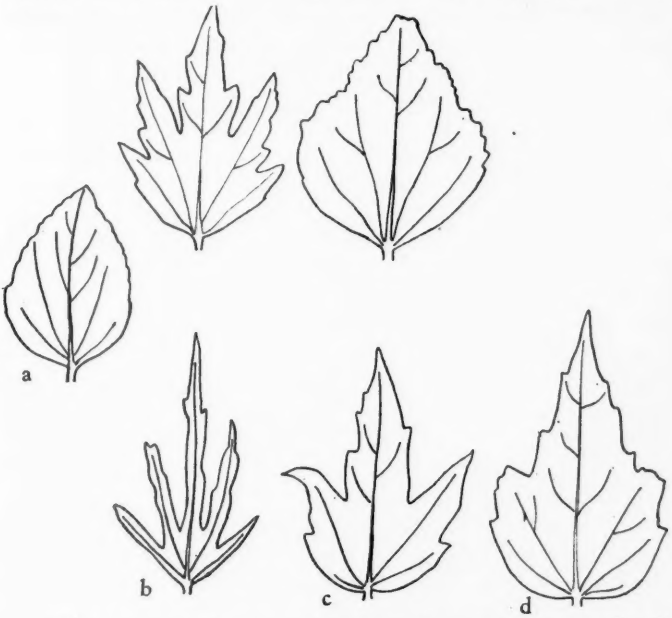

FIG. 1. A comparison of leaf-shape development in "intermediate" (top row) and "narrow" (bottom row) types of *Hibiscus rosa-sinensis*. Primary stage (a); successive stages—juvenile to climax leaf (b), (c) and (d). In a third type the primary stage is retained throughout the life history (see text).

further classified into two types in the juvenile stages, *viz.*, intermediate and narrow, according to the maximum laciniation achieved (see Fig. 1). The family as a whole segregated as follows,

$$3 \text{ entire} : \underbrace{3 \text{ intermediate} : 2 \text{ narrow}}_{2^{\circ} \text{ entire}}$$

which is possibly an approximation to the 1 : 2 : 1 ratio expected on selfing a mono-hybrid. Since "Apricot" is known to be a secondarily entire leaved type, and as Deshpande has shown that

monofactorial leaf shape inheritance occurs in the genus, this is probably the correct interpretation.

The developmental behavior of these leaves is of particular interest since it makes possible a comparison, which transcends the limits of a single genus, between similar developmental mechanisms. In the development of the *Hibiscus* and *Gossypium* leaves, the first three phases are clearly similar, but in the former a fourth phase supervenes which has no true counterpart in the latter. However, in the *Gossypium* system there is one allele (L^R) which produces a climax leaf with accessory lobes, *i.e.*, a five-lobed leaf with an extra lobule on either side of the median lobe (Stephens, 1944). It is possible that given a suitable genotypic background, the action of the L^R allele could be accelerated so as to produce a continuation of development in which the accessory lobes would eventually obliterate the main sinuses (as actually occurs in the *Hibiscus* system). Such a genotypic background is probably not available in *Gossypium*, but it is known that the rate at which the leaf-shape alleles in this genus work is greatly modified by altering the flowering habit (Stephens, in press (b) and (c)). It appears, for instance, that on the late-flowering, perennial background provided by the arboreal species, *G. aridum* (R and S) Skovsted, lobing is retarded and the leaf remains in the primary entire leaf phase. The converse effect, *i.e.*, acceleration of development in leaves already lobed, which would be of more interest in the present instance, is much less easily demonstrated since the breeding of early flowering annual types for commerce has already set a limit to further acceleration. Nevertheless, considerable evidence is available, from developmental observations of species whose leaf shapes have not yet been studied genetically, which supports the thesis of a basic leaf shape mechanism common throughout the Malvaceae. In *Hibiscus esculentus* L. leaf-shape development is of the *Gossypium* type—not of the type found in its sister species, *Hibiscus rosa-sinensis*, and at least two of the climax leaf shape patterns which occur have recognizable counterparts in the cultivated cottons. The leaf-shape patterns of the related genera, *Thespesia*, *Shantzia* and *Gossypioides*, can also be matched by patterns occurring in American diploid and Asiatic species of *Gossypium* (Fig. 2).

It can scarcely be doubted, then, that similar monogenic mechanisms occurring throughout one genus (*Gossypium*) and also in related genera (*Hibiscus* and *Malva*) implies an original homol-

ogy which has been retained through their diverging evolutionary histories. If this is accepted the possibility must also be admitted that the phenotypic expression of a gene may only represent a fraction of its full potentialities, that is, quite apart from considerations of mutability and segregation. It is well known that variability in genetic expression at a single locus does not depend solely on mutation and segregation but may be conditioned by "modification," *i.e.*, by change of balance with genes at other loci. Usually, however, modification is considered a minor source of variability and it is not so often realized that its potentialities may be enormously greater than is commonly observed within the limits of a single species. Were it not for the

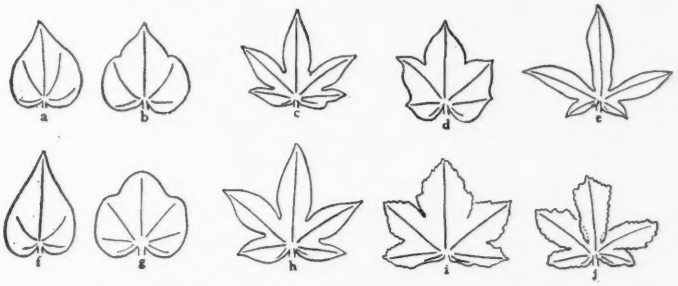

FIG. 2. Comparison of various *Gossypium* leaf shapes (top row) with their phenocopies in related genera (bottom row). (a) *G. aridum* (R and S) Skovsted; (b) *G. harknessii* Brandg.; (c) *G. arboreum* L. (L^1 allele); (d) *G. hirsutum* var. *taitense* Hutchinson (1 ellele); (e) *G. hirsutum* L. (L^0 allele); (f) *Thespesia populnea* Soland; (g) *Shantzia garckeana* Lewton; (h) *Gossypioides bussei* Hutchinson; (i) and (j) *Hibiscus esculentus* L. Leaves reduced to approximately equal sizes for purpose of illustration.

isolating mechanisms which restrict interspecific, and usually prevent intergeneric recombinations, it seems likely that a greatly extended range of modification could be achieved, resulting in the expression of many otherwise latent genetic characters. In the present example it appears likely that the potentiality for producing a secondarily entire leaf, latent in *Gossypium*, is expressed in *Hibiscus*, and it can not be assumed that the final expression has been reached in either genus if it is reached at all! The phenotype provides, as it were, only a cross-section of the genotypic structure, and in each section only a limited range of the individual genes' activities can be observed. Furthermore, it is important to realize that the range of expression can be shifted considerably without altering the genotypic background.

In *Gossypium*, alterations in the photoperiod may exert a considerable effect on the rate of action of the leaf-shape alleles (Stephens, in press (a)) and this provides the clue to the striking leaf-shape modifications observed in *Cannabis sativa* L. by Schaffner (1926). The recent work of Went (1944) suggests that thermoperiodicity may be a still more potent factor in shifting the range of phenotypic expression. A new environment may therefore reveal hidden potentialities, without in the first instance shifting the genotypic balance by natural selection. This "plasticity" is already familiar to experimental taxonomists (Turesson, 1922; Clausen *et al.*, 1940)—its genetic basis is clarified by a developmental approach.

The peculiar course of leaf-shape development in *H. rosa-sinensis*, where an entire leaf becomes laciniated only to return subsequently to the entire condition, suggests a possible explanation of the dissected cotyledons found in certain wild species of *Gossypium*, notably *G. sturtii* F. Muell. Haldane (1932) has drawn attention to the striking possibilities of changing phenotypic expression by altering the timing of gene action. Arguing along similar lines, one might suppose that acceleration of leaf-shape development in *Hibiscus* would throw back the laciniated phase earlier and earlier in the ontogeny of the plant, eliminating eventually the primary entire phase and initiating laciniation in the cotyledons. This situation would resemble superficially that actually occurring in *G. sturtii*, where laciniated cotyledons are succeeded by entire foliage leaves.

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